Investigating the Prevalence of Traditional and Novel Chronic Kidney Disease Risk Factors in a Mixed-Aged, Non-Clinical Population.

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CERTIFICATE OF ORIGINALITY

This is to certify that I am responsible for the work submitted in this thesis, that the original work is my own, except as specified in the acknowledgements and in references, and that neither the thesis nor the original work contained therein has been previously submitted to any institution for a degree.

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Abstract

Chronic kidney disease (CKD) is a common disorder that currently serves as a major global public health issue due to raised risk of cardiovascular disease (CVD), kidney failure, and other complications. With the ever-growing increase of ageing populations and rising global frequency of diabetes and other chronic non-transmissible diseases, present clinical data suggests a corresponding worldwide increase in prevalence of chronic kidney disease and end-stage renal failure (ESRF).

The overall aims of this thesis was to study the prevalence of known traditional and novel CKD related risk factors in a non-clinical population, examine the applicability of several predictive equations in the estimation of GFR for the stratification of CKD, Investigate the relationships between key risk factors amongst different subgroups of the total study population, and Provide evidence in the need for further healthcare screening strategies to help identify early onset CKD.

This project carried out quantitative and qualitative assays to investigate the Incidence of chronic kidney disease risk factors in a non-clinical, mixed-age university population and establish the risk of development of CVD, CKD and type 2 diabetes. Baseline information was reported on a questionnaire and blood serum samples were analysed for biochemical components including blood glucose, cholesterol, triglycerides, creatinine, fructosamine and high sensitive C-reactive protein. Renal status was measured using the Kidney Disease: Improving Global Outcomes (KDIGO) classification. 186 individuals (64% females) were recruited and separated into two age groups (under and over 30 years).

Overall, 39% of participants showed a BMI above 25 with 13% indicating levels above 30. Kidney function data revealed 21% of participants with an estimated glomerular filtration rate below 90ml/min/1.73m^2. Preliminary data suggests a significant difference in diastolic blood pressure, body fat percentage and renal function between the two age groups. Regarding biomarkers of CKD, the dominant finding was a strong relationship between the markers of glycaemic control and a reduced eGFR. Likewise, this relationship was also visible in the lipid markers of the study population.

In summary, the findings from this study in combination with previous reports suggest that traditional biochemical risk markers such as those for diabetes and obesity may share an increased relationship with a reduced kidney function. While we did not see a direct correlation between the non-traditional marker, hsCRP, it was possible that this was a consequence of insufficient population numbers.

Investigation of renal equations provided evidence of a greater accuracy in the measurement of estimated kidney function using the newer equation system, CKD-EPI, in comparison to clinically regarded formulas such as Cockcroft-Gault and MDRD.

When evaluated on an additive scale, the modification of these variables by the presence of other risk factors has important clinical and public health implications with respect to CKD case findings and mass screening strategies.

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Chapter 1.0
Introduction

1.1 Background

Chronic kidney disease (CKD) is a common disorder that currently serves as a major global public health issue due to raised risk of cardiovascular disease (CVD), kidney failure, and other complications (El Nahas, 2005 and Oh *et al.,* 2010). With the ever-growing increase of ageing populations and rising global frequency of diabetes and other chronic non-transmissible diseases, present clinical data suggests a corresponding worldwide increase in prevalence of chronic kidney disease and end-stage kidney failure (James *et al*., 2010). Due to the progressive nature of CKD and subsequent end-stage renal disease (ESRD), considerable pressure is being placed around the quality and availability of global healthcare resources (El Nahas and Bello, 2010). Despite the vast number of risk profilers and related therapies available to clinical patients, the suboptimal status of cardiovascular (CV) and renal protection still remains high in healthcare settings. Many reasons for this are indicated by the progression to ESRD or CV events in patients who receive optimal treatment, thus suggesting the need for newer, better predictors or, more importantly, new therapy targets (Zeeuw *et al.,* 2005).

1.2 Functions of the Kidney

The kidney is a complex organ which is key to many of the functions that take part in the body. It removes a number of toxins, excess salts and waste products through filtering of the blood as well as controlling the balance of water and pH levels and regulating blood pressure (Levey *et al.,* 2003). Each kidney consists of around one million nephrons, each containing glomeruli, capillaries, arterioles, and tubules. Regulation of blood pressure is carried out through a number of ways, this includes the renin-angiotensin aldosterone system (RAAS) which acts to maintain blood pressure and fluid homeostasis through the constriction of vessels and retention of water and minerals (Coresh *et al.,* 2005). The kidneys also act as an endocrine organ by producing erythropoietin, which controls the production of red blood cells. Furthermore, the kidneys also play a role in the calcium-phosphorus metabolism process whereby calcium is reabsorbed in the tubular system. In cases such as CKD, calcium levels become depleted, thus the kidneys activate a compensatory increase in parathyroid hormone (PTH), resulting in the stimulation of vitamin D to facilitate the absorption of calcium from the small intestine and increase calcium and phosphate efflux from bone (Sahay, 2012).

1.3 Definition, classification and staging

Unlike most diseased group patients, those with CKD do not share a common underlying diagnosis and as a result, the pathophysiology and natural history may differ significantly

between same-group individuals. However, it was acknowledged by the authors of the guidelines that these patients did have one thing in common; decreased kidney function due to a chronic disease process. They also established the variance in markers of impaired kidney function in differing diagnoses (Ikizler, 2009). However, despite these observations, once kidney disease is established the features that define CKD apply across disease states and comprise evidence of damaged renal parenchyma as demonstrated by active urinary sediment and/or structural abnormality (must be present for stages 1 and 2) and/or evidence of decreased kidney function as demonstrated by a reduced glomerular filtration rate (GFR) and chronicity to distinguish it from acute kidney injury (AKI) (Parr and Siew, 2014).

Worldwide, the growing number of patients with ESRD is continuing to act as a burden amongst populations despite the recent advances in our understanding of the uraemic state and improvements in the science and technology of renal replacement therapy (Ikizler, 2009). Over 1 million people are estimated to suffer from ESRD in the world today (NxStage Medical, 2012). These findings have led the scientific community, now over a decade ago, to identify CKD as an 'important public health problem' which emphasises the need for timely diagnosis and treatment for prevention of ESRD (Ikizler, 2009). The definition and classification of CKD guidelines were first introduced by the National Kidney Foundation (NKF) Kidney Disease Outcomes Quality Initiative (KDOQI) in 2002 (National Kidney Foundation, 2002), and later adopted internationally by the Kidney Disease: Improving Global Outcomes (KDIGO) initiative to guide identification of cases and facilitate management (Levey *et al.,* 2005). Subsequently, these CKD guidelines have helped shift the concept of kidney disease from that of an uncommon life-threatening condition that requires care by clinical nephrologists to that of a common condition with a range of severity and thus warranting attention by general internists and demanding the application of tactics for early diagnostic measures, preventative strategies and managerial procedures (Levey *et al.,* 2009).

In general, CKD is defined as "structural or functional abnormalities of the kidney that persist for at least 3 months and are manifested by either kidney damage (most frequently detected as persistent albuminuria; >30 mg albumin/g creatinine) or a sustained reduction in glomerular filtration rate (GFR) (<60 mL/min per 1.73m^2)" (Levey *et al.,* 2009). The recommended screening methods for detection of CKD involves the use of 'untimed urine checks' for the presence of proteinuria, measuring the albumin:creatinine ratio (>30 mg/g as designated cut-off point), ultrasonographic images (e.g., cysts in adult polycystic kidney disease), and/or total protein:creatinine (>200mg/g) (NICE, 2014). Assessment of kidney function is often carried out through either the direct measurement of GFR (mGFR), using urinary or plasma clearance of

exogenous filtration markers such as inulin, or most frequently through the estimation of GFR (eGFR) via serum creatinine levels (Stevens and Levey, 2009).

To avoid the misclassification of individuals based on serum creatinine alone, a number of well-known eGFR equations have been developed which include additional features such as age, sex, ethnic origin, and body size (Slee, 2012). The Cockcroft-Gault equation, which was introduced in 1976, was subsequently used by many clinical services in routine practice for the determination of eGFR (Cockcroft and Gault, 1976). Although the formula is still currently in use for purposes such as dose adjustments for renal clearance-related drugs, it presents as a complex formula which requires anthropometric measurements that may not be routinely available, and in some cases, has been known to overestimate GFR (Levey *et al.,* 1999).

The modification of diet in renal disease (MDRD) study equation is one of the most well-known and clinically embedded formulas currently in use. Developed by Levey et al in 1999, the MDRD equation provides an estimation of GFR using patients' serum and other readily available data. However, likewise to the Cockcroft-Gault formula, much criticism has been called out by researchers on the accuracy of the MDRD equation, with many arguing that this formula has the tendency to underestimate in individuals who present a GFR above 90 ml/min/1.73m^2 (Murata *et al.,* 2011). The potential risk and severity of CKD using the five-stage scheme is based predominantly on GFR (**table 1.1**), however risk of complication at a given rate can be altered considerably by the amount of proteinuria produced by the patient.

Since the concentration of creatinine in serum alone is insensitive to early disease, identification and staging of chronic kidney disease on the basis of estimated glomerular filtration rate was an important advance that was able to facilitate both research and clinical care. Nonetheless, controversy continues to surround the existing classification system, specifically with regard to its propensity to overestimate prevalence; its failure to fully incorporate prognostic information from proteinuria; and the potential for misclassification of some people as having CKD in the absence of clinically relevant kidney disease (James *et al.,* 2010).

Table 1.1 Classification of chronic kidney disease stages 1-5

Stage	Description	GFR (ml/min/1.73 m^2)	Clinical Presentations
-	At increased risk	$\geq$ 60 (without markers of damage)	CKD risk factors

1	Kidney damage with normal or increased GFR	≥90	Markers of damage (nephrotic syndrome, nephritic syndrome, tubular syndromes, urinary tract symptoms, hypertension due to kidney disease)
2	Kidney damage with mildly diminished GFR	60-89	Mild complications
3a	Mildly to moderately decreased GFR	45-59	Moderate complications
3b	Moderately to severely decreased GFR	30-44	
4	Severely decreased GFR, with or without other evidence of kidney damage	15-29	Severe complications
5	Kidney failure	<15 (or dialysis)	Uraemia, Cardiovascular disease

CKD is defined as either kidney damage or GFR <60 mL/min/1.73 m^2 for ≥3 months. Kidney damage is defined as pathologic abnormalities or markers of damage, including abnormalities in blood or urine tests or imaging studies. (Adapted from: KDIGO CKD Work Group, 2012. **Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease.** Table 1.2.3).

1.4 Epidemiology

Evidently, it cannot be ignored that CKD prevalence across the world is growing at a substantial rate. With an estimated global disease prevalence of between 10% to 16% in adults and an annual growth rate of 8% in ESRD incidence, figures indicate a vastly growing issue surrounding the renal health status of many populations (Chan, 2012). As CKD is often asymptomatic, studies to determine its prevalence must rely on community-based screening. As **Table 1.2** shows, there is considerable variation in prevalence depending on the methods used and the populations studied (Evans and Taal, 2011). In the UK, the HSE 2010 study provided data from more than 6,000 participants in which 49% of men and 52% of women were reported to have abnormal eGFR levels (below 90ml/min/1.73m^2). Nonetheless, a much lower proportion of individuals were described as having an eGFR below 60ml/min/1.73m^2 (6% of men and 7% of women). Most apparent from this is the gradual decrease in eGFR with increasing age in both genders, from 1% of men and fewer than 1% of women aged 16-24 to 28% of men and 35% of women aged 75 and over, as illustrated in **figure 1.1** (Roth *et al.,* 2011).

Table 1.2 CKD prevalence in a range of populations

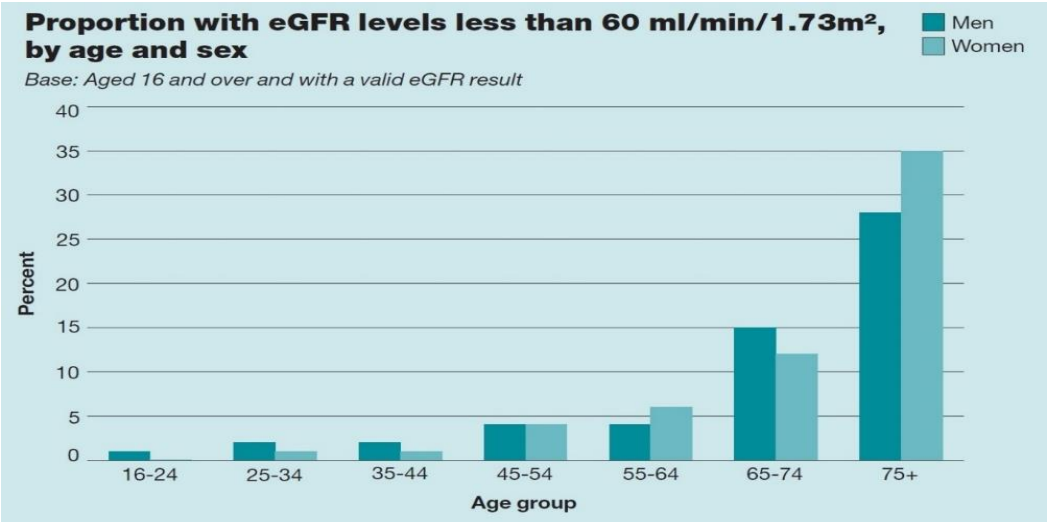
Country	Study	Subject population	Age of subjects	Definition of CKD
Australia	AUSDIAB	11,247	≥25	CKD 1-5
D.R. of Congo	Sumaili et al	503	≥20	CKD 1-5
Norway	HUNT 2	65,604	≥20	CKD 3-5
South of China	Chen et al	6,311	≥20	CKD 1-5
UK	HSE 2010	6,000	≥16	CKD 1-5
USA	NHANES IV	13,233	≥20	CKD 1-4
Pakistan	Jessani et al	2,873	≥40	CKD 3-5
Eastern China	Xue et al	14,399	≥18	CKD 1-5
Nigeria	Stanifer et al	5,005	≥20	CKD 1-5
Republic of Ireland	Austin et al	278,630	≥18	CKD 3-5

(Adapted from: Evans and Taal, 2011. **Epidemiology and causes of chronic kidney disease.** Table 1).

Although we have little information on the total burden of CKD in the UK, data from other studies such as the National Health and Nutrition Examination Surveys (NHANES) in the USA not only gives likely overall population prevalence, but also suggests that the prevalence is increasing (Coresh *et al.,* 2007). Comparison between the prevalence of CKD in NHANES 1988-1994 with NHANES 1999-2004 showed a significant increase in population prevalence from 10.03% to 13.07% (National Collaborating Centre for Chronic Conditions, 2008). Many researchers suggest that this rising prevalence in CKD has been brought about by the current escalation in CKD risk factors including obesity, hypertension and most notably the rise in ageing populations across America and much of the world; the incidence of ESRD is much higher in elderly people than in the general population (more than 1200 per million in over 65's in America) (El Nahas and Bello, 2005). Despite this remarkable rise in related risk factors, one disease continues to make itself known amongst renal sufferers across the globe, that is, type 2 diabetes mellitus (T2DM). Recent estimates suggest that T2DM accounts for 382 million sufferers worldwide, and as a result, diabetic nephropathy is now one of the leading causes of ESRD, accounting for 30-40% of all kidney-related diseases (El Nahas and Bello, 2005). This noticeable rise in incidence levels has been echoed by the United States Renal Data System (USRDS) findings, which demonstrates a rapid rise in ESRD cases with diabetes listed as the

primary cause since the 1980's (Atkins, 2005). Although, recent figures now show a gradual decline in numbers from 2010-2012 (**Figure 1.2a**). Similarly, the rate of new ESRD cases has declined since 2006, with the lowest rate in 2012 since 1997 (**Figure 1.2b**).

Figure 1.1 Number of individuals with an eGFR below 60ml/min/1.73m²

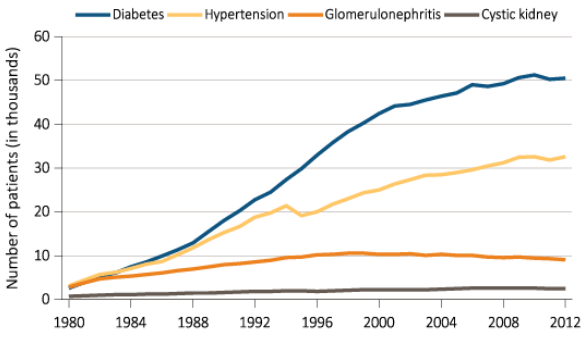


(Adapted from: Roth et al., 2011. **Kidney disease and renal function.** Figure 8C).

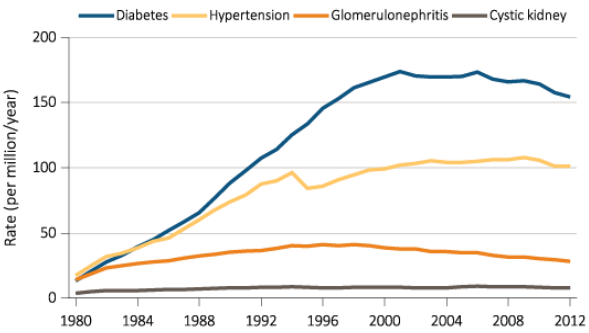
Studies have also begun to highlight some of the striking differences in the CKD risk attributed to diabetes and hypertension amongst various ethnic groups. Compared to Caucasians, the prevalence of diabetic renal failure is two- to threefold greater in blacks, Asians and Hispanics, and up to 18-fold greater in Native Americans. Black race/ethnicity is also associated with more rapid progression of renal disease (Daugirdas, 2011). In the UK, these findings prove to be evident in people of south Asian ethnicity, where CKD stage 5 is approximately three times more frequent than in those of white ethnicity, mainly due to increased incidence of diabetes as well as renal dysplasia and almost all types of glomerular disease (Higgins, 2009). In the USA, the CARDIA study displayed a 2.4-fold increase of renal

Figure 1.2 (a) Trends in ESRD Incident cases, and (b) Rate of new ESRD cases per million/year, by primary cause of ESRD, in the U.S. population, 1980-2012).

(a) Number of CKD cases



(b) Rate of CKD incidence



(Retrieved from: Saran et al., 2015. **US Renal Data System 2014 annual data report: epidemiology of kidney disease in the United States.** Figure 1.7).

impairment odds in women and a ninefold increase in men when compared with Caucasians (Stehman *et al.,* 2003).

However, whilst the magnitude of CKD has been better defined in developed countries, increasing evidence indicates the burden of CKD is as great or even greater in developing countries. Disadvantaged communities, such as those from low resource, racial, and ethnic minorities and/or indigenous backgrounds, suffer from marked increases in the incidence, prevalence and complications of CKD (Kimmel and Rosenberg, 2015). In India, a population-based study presented diabetic nephropathy to be the most common cause of ESRD, occurring in 44% of individuals. Findings also determined the average crude incidence rate of ESRD to be 150 per million population and where CKD equalled cardiovascular disease as a cause of death in people with diabetes (Modi and Jha, 2006).

The fact that even in developed countries racial and ethnic minorities display a disproportionate burden of the disease suggests there may be multiple other elements beyond traditional risk factors associated with CKD.

One of the largest concerns surrounding CKD prevention is in fact disease awareness. Findings from the Kidney Early Evaluation Program (KEEP), a community-based study, revealed only 10% of the 26,213 participants were actually aware of suffering from CKD. Awareness levels were particularly low however in those with early CKD, with 5.1%, 6.3% and 10.0% for stages 1 to 3. Alternatively, almost 40% of stage 4 and 60% of stage 5 sufferers were aware of having kidney disease (Vassalotti *et al.,* 2010).

1.5 Risk factors of CKD

Previous clinical and epidemiological research has shown key associations between numerous factors as well as the initiation and progression of CKD. Fundamentally, two well defined groups have been established: those that are causal to CKD (risk factors) and those that are associated with CKD in the absence of established causal relations (risk markers) (El Nahas, 2005). Presently, the relationship of these biomarkers and as to whether they are risk factors or risk markers for the progression of CKD is not well understood. Risk markers are not causally involved in CKD progression, however they may indicate the probability of progression and therefore could potentially be used as diagnostic tools. Risk factors causally affect disease progression and are therefore interesting therapeutic targets (Kronenberg, 2015).

1.5.1 Aetiology and risk factors

Progression of most kidney diseases can be seen to take more than 10 to 15 years, initially without any symptoms. This can prove a difficult task when trying to identify the aetiology of disease. Many indications show that clinical and sociodemographic risk factors play a significant impact on the susceptibility and initiation of chronic kidney disease; this includes a higher risk in elderly populations (Stevens *et al.,* 2010); those that have a family history of CKD such as autosomal dominant polycystic kidney disease (ADPKD) (Levey *et al.,* 2003); racial factors as seen in Afro-Caribbean and Asian individuals and those that have a pre-existing disease such as diabetes mellitus, metabolic syndrome and cardiovascular disease (El Nahas and Bello, 2005).

Established or suspected clinical and sociodemographic risk factors associated with the susceptibility or initiation of CKD are shown in **Table 1.3**:

Table 1.3. Risk factors associated with the initiation or susceptibility of chronic kidney disease

Age	Nephrotoxins
Gender	Primary kidney disease
Ethnicity	Urological disorders
Family history of CKD	Cardiovascular disease
Diabetes Mellitus	Dyslipidaemia
Metabolic syndrome	High normal urinary albumin excretion
Hyperfiltration state	Low birth weight

Adapted from: Owiredu *et al.,* 2012. **Metabolic syndrome among Ghanaian patients presenting with chronic kidney disease.**

1.5.2 Progression of CKD

The progression of established CKD is variable and depends on several risk factors or markers. Progression factors can be separated into two defined groups; traditional and novel **(Figure 3.1).** Traditional factors includes those that are already classically established towards the progression of CKD such as obesity, ethnicity, smoking, hypertension, and the presence of proteinuria (Kronenberg, 2009). Metabolic risk factors have also been implicated in the progression of CKD; this was made evident by the UK Prospective Diabetes Study in 2003 which identified the acceleration of diabetic nephropathy in type 1 and type 2 diabetes (Adler *et al.,* 2003).

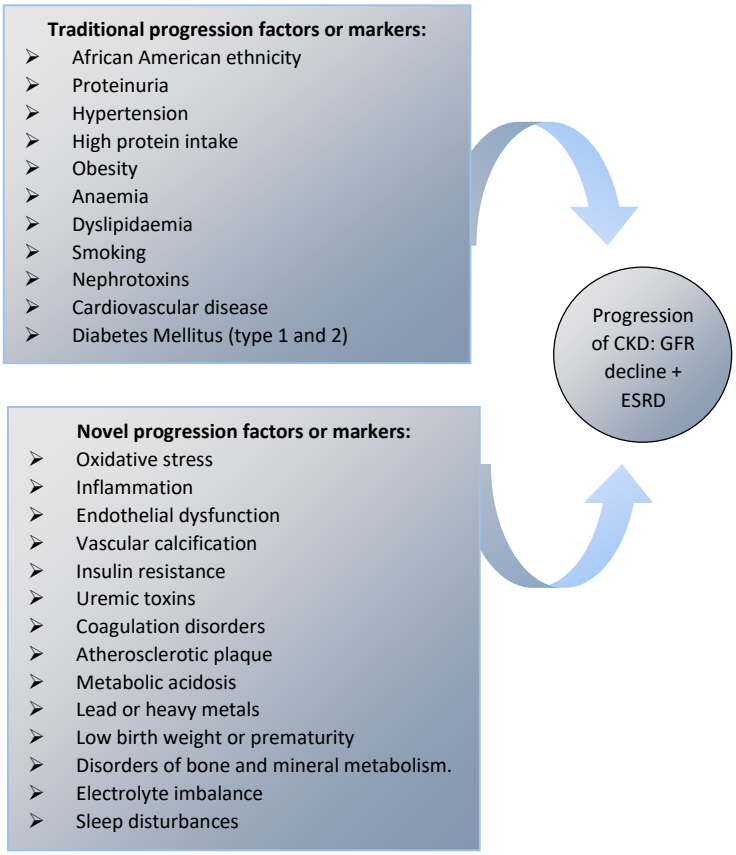
The severity and extent of renal complications in patients with CKD is disproportionate to the number and severity of traditional risk factors. As a result, this realisation has focused the importance of non-traditional risk factors including reduced haemoglobin levels, microalbuminuria, increased inflammation and oxidative stress, and abnormalities in bone and

mineral metabolism. Nonetheless various studies in patients with advanced CKD have not shown that normalisation of these non-traditional risk factors improves survival rates. Moreover, the mechanisms by which these novel risk factors contribute to CKD risk are largely unexplored (Kendrick and Chonchol, 2008).Therefore, although current treatment of patients with CKD includes management of traditional and nontraditional risk factors, the value of modifying some nontraditional risk factors remains unclear.

Hypertension

With a global prevalence estimated at nearly 1 billion people, high blood pressure (defined as >140/90 mm Hg) is labelled as one of the major leading risk factors for development and progression of CKD (Bakris and Ritz, 2009). The aging population of the world coupled with increased prevalence of obesity and type 2 diabetes provides a platform for increased levels of hypertension within populations; so much so that figures have been estimated to rise to 1.56 billion sufferers by 2025 (Kearney *et al.,* 2005). For many years now hypertension has been established as both a cause and consequence of CKD (Klag *et al.,* 1996; Jafar *et al.,* 2003) and is also shown to increase the risk of ESRD (Chapman *et al.,* 2010). Supported theories for the suggested development of hypertension in CKD includes the activation of the renin angiotensin aldosterone (RAAS) pathway, abnormal mineral metabolism which effectively leads to arterial stiffness, sympathetic activation, and alterations to water and salt handling with sodium and water retention (Chan, 2012). Aside from these causes, studies have also indicated a strong relationship between blood pressure and proteinuria in hypertensive CKD patients; this was made evident from the Modification of Diet in Renal Disease (MDRD) study whose findings indicated the importance of hypertension control in patients with proteinuria higher than 1 g/day. By lowering blood pressure to a target of

Figure 1.3 Traditional and novel risk factors for the progression of chronic kidney disease.



(Adapted from: Kronenberg, 2009**. Emerging risk factors and markers of chronic kidney disease progression.** Figure 1).

125/75 mm Hg, a greater decrease was achieved in the rate of decline of GFR than in those with less proteinuria (Klahr *et al.,* 1994). Likewise, it can also be said that proteinuria is exacerbated by frequently increased blood pressure, resulting in the promotion of tubular atrophy, fibrosis, and tubulointerstitial inflammation and thus further elevating BP (Toto, 2005).

Dyslipidaemia

Irregularities in lipid metabolism, coupled with alterations in lipid profile, are seen to be a common occurrence in patients with CKD. These abnormalities tend to occur in the earliest stages of kidney disease and consequently lead to a decrease in glomerular function (Kowalski *et al.,* 2015). Studies investigating the disorder of lipid metabolism in CKD patients have identified a significant increase in triglycerides (TGs), total cholesterol and low density lipoproteins (LDL) (Parmar *et al.,* 2014). Reasons for this are suggested by the down regulation of lipoprotein lipase and abnormalities within the lipoprotein profile which ultimately leads to a reduction in receptor binding and lipoprotein uptake. Additional metabolic defects result in an increase of very low density lipoproteins (VLDL) and apolipoprotein B (ApoB)-rich lipids (Kaysen, 2006). Combined with a decrease in high density lipoproteins (HDL), these defects prove to generate a highly atherogenic profile within CKD patients and therefore vastly escalate the risk of CVD outcome (Tannock, 2015). Considering the fact that patients with CKD are amongst the highest risk for CV-related events, the decision to implement specific recommendations regarding dyslipidaemia in CKD patients was put forward by the Kidney Disease Outcomes Quality Initiative (K/DOQI) (The National Kidney Foundation, 2003). The outcome suggested a strong agreement towards the management and importance of dyslipidaemia in patients with kidney disease. However, recent trials have displayed only a modest benefit in dyslipidaemia treatment with regards to cardiovascular mortality, therefore indicating a need for further study (Afsar, 2013).

Diabetes mellitus

In the UK alone, over 3.2 million people are affected by diabetes, equating to 6% of the total population (Diabetes UK, 2015). Diabetes has long been regarded as the most common single cause of end-stage renal disease in both the US and Europe (Molach *et al.,* 1998). A considerable increase in diabetes prevalence amongst populations, mainly due to the rising trend in lifestyle habits and westernised cultures, has meant that diabetic nephropathy is now more common than ever; with 40-50% of type 1 and type 2 diabetic patients being affected (Gross *et al.,* 2005). Increased risk of death, mainly from cardiovascular causes, is present in diabetic patients with

microvascular disease and is defined by increased urinary albumin excretion (UAE) in the absence of other renal diseases. Morrish and colleagues reported that kidney disease accounted for 21% of deaths in type 1 and 11% of deaths in type 2 diabetes (Morrish *et al.,* 2001). Early indications of nephropathy are marked by the presence of abnormal albumin levels within the patient's urine (>30mg/day), also known as microalbuminuria (**Table 1.4**) (Bennett and Aditya, 2015). Without specific interventions, progression to overt nephropathy, or macroalbuminuria is heralded by a UAE of >300mg/day, sufficiently leading to a progressive decline in glomerular function rate and hypertension (Gross *et al.,* 2005). Initially, studies from the 1980's had demonstrated that around 80% of microalbuminuric type 1 diabetic patients progressed to proteinuria over a period of 6-14 years (Mogensen and Christensen, 1984). However, in more recent times, studies have showed only 30-45% of proteinuria progression in these patients over 10 years (Caramori *et al.,* 2000). Suggestions are favoured by the more intensive glycaemic and blood pressure control strategies that have been implemented over the years.

Table 1.4 Interpretation of various proteinuria measurement tests

	Normal	**High**	**Very high**
Albumin creatinine ratio (mg/mmol)	< 3	3-30	>30
Urinary albumin excretion (mg/day)	< 10	10-300	>300
Urine diptest	-ve to trace	Trace to 1+	>1+

Adapted from: Stringer, 2013. **Establishing a high risk CKD cohort: Cross-sectional analysis and early outcomes.** Table 2.

With regards to current diagnostic and prognostic strategies for type 2 diabetes, there are two tests which are commonly employed in the healthcare background; this includes the measurement of plasma glucose and glycated haemoglobin (HbA1c). Nevertheless, it goes without saying that these measures do not prove to be fool proof due to the biased nature of a number of clinical and analytical factors. Therefore, the introduction of other indices of glucose homeostasis in clinical practice such as fructosamine and glycated albumin (GA) may be regarded as a favourable alternatives, especially in those individuals that HbA1c may prove to be biased or even unreliable (Danese *et al.,* 2015). Such patients include those with rapid changes of glucose homeostasis, individuals with red blood cell disorders and most importantly, in those with renal disease. In addition, further advantages of fructosamine and GA over HbA1c are represented by the lower reagent costs and being able to automate the biochemical analysis on many conventional laboratory instruments (Dingari *et al.,* 2012).

As a consequence of the greater susceptibility to glycation of albumin and other plasma proteins compared to intracellular proteins such as haemoglobin, the blood levels of fructosamine exhibit a broader fluctuation than those of HbA1c, thus allowing an earlier detection of rapid changes of blood glucose. Accordingly, the measurement of fructosamine seems useful not only as an alternative index of glycaemic control in conditions in which HbA1c is unreliable , but also for identifying impaired control of blood glucose before any noticeable changes in HbA1c may occur (Roohk and Zaidi, 2008).

Lifestyle factors

It has long been known that lifestyle plays a significant role in the development and progression of CKD (Hallan *et al.,* 2006). Diet, obesity and sedentary lifestyles have been recognised to be contributors towards the development of CKD and associated comorbidities. However it must be said that existing data of the link between dietary protein intake as an independent risk factor for the initiation and/or progression to CKD is sparse (Stengel *et al.,* 2003). Nevertheless, studies into this contributing factor have consistently demonstrated a link between dietary protein intake and systemic blood pressure. One example was seen by De Miguel *et al.,* 2011 whose research saw rats fed a high-protein (30%) diet from 5 to 12 weeks of age. The results produced significant data showing the highest increase in mean arterial blood pressure and urine albumin-to-creatinine ratio when compared to normal and low-protein diets.

Amongst the other lifestyle factors, physical inactivity leading to low exercise capacity is common in CKD patients and particularly those undergoing haemodialysis (HD) (Manfredini *et al.,* 2012). Although a scarcely investigated focus, a recent study examining 'Physical activity and energy expenditure in haemodialysis patients', revealed a compatibility between the physical activity parameters of HD patients and a sedentary lifestyle. It was reported that inactivity in these patients was worsened by the presence of ageing, diabetes and higher BMI, thus suggesting a greater need for improved health training exercises and interventions in CKD patients (Avesani *et al.,* 2012).

Several epidemiological studies have attempted to identify the role of smoking and alcohol consumption in the development of CKD (perneger *et al.,* 1999. Vupputuri and Sandler, 2003). Unsurprisingly, excessive alcohol consumption related to a four-fold risk in the development of renal dysfunction (Perneger *et al.,* 1999). Reasons for this are suggested by the initiation or promotion of atherogenic risk factors such as hypertension, hyperuricaemia, insulin resistance and diabetes (Stengel at al., 2003). A study investigating the joint exposure of heavy drinking

and smoking in >4000 individuals found a five-fold association with the development of CKD when compared with their absence. Clearly the harmful effects from both smoking and drinking are demonstrated as potential factors towards the progression of renal failure. However, despite these findings recent studies have proven contradictory to this assumption; an investigation by Funakoshi *et al.,* in 2012 showed an inverse association between the frequency of drinking alcohol and CKD in healthy Japanese men. Results showed that although regular alcohol consumers showed signs of increased hypertension to non-drinkers, this group had a lower prevalence of CKD and hyper-LDL-cholesterolaemia, as well as increased HDL-cholesterol and a lesser prevalence of diabetes to the non-drinkers group. Although not fully understood, suggestions are targeted towards the protective effect of alcohol by the mediation of increased HDL-cholesterol. (Schaeffner *et al.,* 2005).

The rising trend amongst obese populations is a common yet serious implication to today's health system. With a reported prevalence of around a quarter of adults (26% of men and 24% of women) in the UK (Craig and Mindell, 2014), it is suggested that obesity may be a primary contributor towards the progression of CKD and ESRD (Mount *et al.,* 2015; Tanner *et al.,* 2012). However, it goes without saying that much of the additional risk for CKD observed in those with obesity is associated with the increased prevalence of type 2 diabetes and/or hypertension (Ejerblad *et al.,* 2006). Early observations of this relationship between obesity and CKD can be seen from the Framingham heart study cohort in 1999. In this study, they reported increased levels of body mass index (BMI) which conferred with a higher risk of CKD, as detected by an elevated serum creatinine level (Culleton *et al*., 1999). Similar results were yielded by (Hsu *et al.,* 2006) who found subjects with a BMI of more than 40kg/m^2, i.e. severe obesity, were at a seven fold higher risk of CKD than those in the standard risk group with a normal BMI.

The mechanics behind the exact cause of obesity-induced CKD are highly unexplored and extremely convoluted (Mallamaci and Tripepi, 2013). Ideas have been put forwards to suggest an interplay between the onset of inflammation, insulin resistance, hypertension and dyslipidaemia (Teta, 2010). More specifically, research has shown that a higher visceral fat is linked to increased levels of fasting plasma insulin and triglycerides, resulting in obesity-related glomerulopathy (Mount *et al.,* 2015).

Inflammation

The rate of mortality is higher than expected in patients with CKD which can not only be explained by traditional risk factors such as diabetes, hypertension and dyslipidaemia but by

other factors including inflammation and predisposition to infection which are believed to have a significant contribution towards renal dysfunction and associated cardiovascular comorbidities (Chade *et al.,* 2005). A range of inflammatory markers such as C-reactive protein and fibrinogen have been proposed as risk factors for both CKD and CVD (Rifkin and Sarnak, 2009), many of which are non-modifiable or causal, although recent studies show that treatment of inflammation in patients with increased C-reactive protein may in fact lower the risk of CVD and slow progression of kidney disease (Heidari, 2013). Moreover, the recent development of high-sensitive CRP (hsCRP) has been known to indicate future vascular events in CKD patients, even in those with no previous history of CVD, therefore recommending this globular indicator as a more accurate and sensitive detector of inflammatory state (Kumar and Shobharani, 2015).

Glomerulosclerosis as a result of renal tissue scarring has been proposed to greatly influence the outcome of CKD as this leads to the influx of monocytes, production of lipid-laden macrophages, increased presence of cholesterol and matrix expansion, resulting in fibrosis. Biological studies suggest that inflammation plays a key role in the process of glomerulosclerosis and subclinical measures of the disease have been associated with kidney dysfunction. CKD along with signs of inflammation are commonly found together, with a high prevalence of C-reactive protein, fibrinogen and white blood cell count, and low albumin found in stages 3-5 CKD (Rifkin and Sarnak, 2009).

Oxidative stress

Amongst the many factors that affect the speed of renal decline, oxidative stress has been identified as a powerful effector on progression of CKD (Small *et al.,* 2012). The onset of oxidative stress occurs due to disparities between oxidant formation and insufficient amounts of antioxidants as a defence mechanism of the body. Aside from playing a role in the link between CVD and CKD through inflammatory mechanisms and endothelial injury, oxidative stress is also responsible for the excessive production of angiotensin II, hyperglycaemia and proteinuria which causes further progression of CKD (Putri and Thama, 2014). In addition, the capabilities of oxidants allow for a varied number of cell components to become oxidised such as carbohydrates, proteins, lipids and nucleic acids which, as a result, induces further damage in the kidneys (Kao *et al.,* 2009).

In terms of biological risk markers for oxidative stress there are several oxidative products which have been highlighted as advantageous in the detection of disease, this includes oxidised-LDL, advanced glycosylation end products, and oxidised thiol components which have been known

to contribute towards the pathogenesis of CVD and inflammation in CKD patients with uraemic state (Sung *et al.,* 2013).

CVD in CKD

For many years, the risk association between both cardiovascular and chronic kidney disease has been well established (Foster *et al.,* 2008). Most importantly, findings suggest that estimated cardiovascular mortality rates are between ten to one hundred times higher in late-stage CKD patients when compared to sex-matched individuals in the general population (Thomas *et al.,* 2008). Despite little investigation into early-stage renal failure, recent studies have supported the hypothesis that CVD risk is also prominent in the initial stages of renal decline, thus reinforcing the need for earlier interventions in the healthcare system to help prevent and treat CVD in this high-risk population (Wright and Hutchison, 2009). Furthermore, there has been numerous documented studies which highlight the prevalence of cardiovascular risk factors in the general population which contribute towards CVD risk in CKD patients (Yamamoto and Kon, 2009). In fact, the number of Framingham risk factors specific to CKD patients, far exceeds those with a normal renal function.

Hypertension is a traditional cardiovascular risk factor which contributes towards the cardiovascular risk associated with CKD (Tedla *et al.,* 2011). In 2005, Muntner and colleagues demonstrated the enhanced risk of new or recurrent cardiovascular events in individuals with stage 2-3 CKD. Interestingly, their findings displayed a much higher risk association between systolic blood pressure and cardiovascular death than both diastolic blood pressure and heart rate (Muntner *et al.,* 2005). In contrast however, some reports have shown a U-shaped relationship which exists between systolic blood pressure and mortality, with high or low blood pressure indicating increased mortality rates in stage 5 CKD patients (Myers *et al.,* 2010).

Aside from hypertension, there is also well documented findings on the association between CVD risk and diabetes mellitus in CKD patients (Kanda *et al.,* 2008). Moreover, in those with moderate to severe renal dysfunction, a lower fasting plasma glucose and/or glycated haemoglobin, correlates with an overall lower risk of all-cause mortality and a reduction in cardiovascular death (Suckling and Gallagher, 2012). A common complication which resides in CKD patients is the presence of left ventricular hypertrophy (LVH), this frequent illness is also deemed a cardiovascular risk determinant in those with reduced renal function and is thought to be initiated by preceding cardiorenal risk factors such as hypertension and anaemia (Di Lullo *et al.,* 2015).

Although many of the traditional Framingham risk factors are present in those with CKD, there are also several novel cardiovascular risk markers that are unique to this specific population. This includes anaemia which is known to occur adverse cardiovascular outcomes in CKD patient (Virani *et al.,* 2008). In addition, irregular serum phosphate levels, calcium-phosphate ion product, and parathyroid hormone levels are also regarded as independent CV risk factors in late-stage renal dysfunction (Thomas *et al.,* 2008). Increased calcium-phosphate products as well as the aggregated dosage of oral calcium-based phosphate binders strongly correlates with the range and progression of arterial calcification in those with stage 3 and 4 CKD, thus resulting in clinical morbidity and mortality in these patients (Friedman, 2006).

Likewise, inflammation is regarded as playing a role in mediating CV risk in in CKD (Silverstein, 2009). Elevations in the markers of inflammation are often seen in CKD patients, with some studies showing correlations between C-reactive protein (CRP) and predictions of cardiovascular outcomes in CKD patients (Bazeley *et al.,* 2011). A study by Menon *et al,.* carried out the investigation of samples from the Modification of Diet in Renal Disease study patients, involving the measurement of CRP concentration and analysing the relationship between long-term outcomes. During a 10 year median follow-up period, their findings showed all-cause mortality to be existent in 20% of patients and CVD mortality in 10%. Additionally, Increased CRP levels were shown to independently predict all-cause and CV mortality after adjusting for cofounding variables. Therefore leading to confirmation from the authors that elevated CRP levels are useful in the prediction of outcomes in CKD patients (Menon *et al.,* 2005).

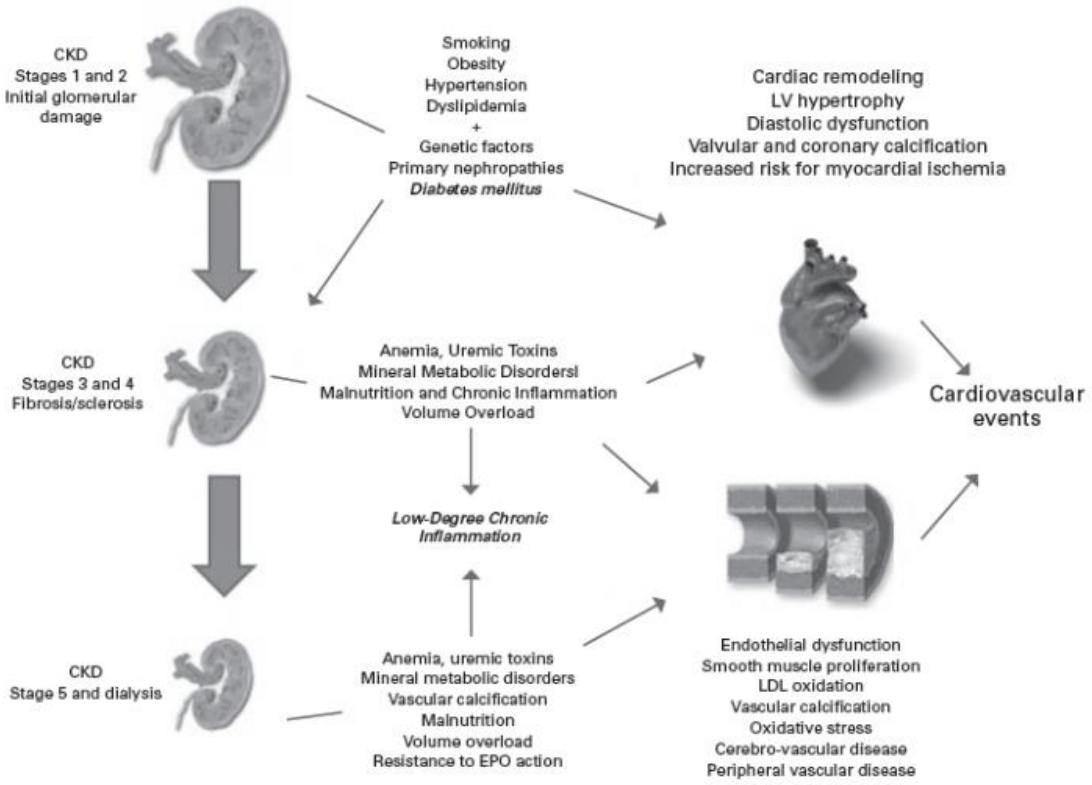
1.6 Pathophysiology

The pathophysiology of CKD is complex and in large part dependent on the primary cause (Parmar, 2002). After a primary acute or chronic insult occurs, such as in diabetic nephropathy or lupus nephritis, many common pathways are activated to perpetuate glomerular and tubulointerstitial injury (Bird and Walker, 2015).

In the initial stages of CKD, the traditional risk factors for CVD act as triggers not only for initiating the deleterious modifications in the cardiovascular system, but also as promoters of CKD progression (**Figure 1.4**). In intermediary stages of the disease, the typical CKD phenomena involved in the pathogenesis of CVD, such as anaemia, mineral metabolic disorders, and systemic inflammation begin to install. In the later stages of CKD and dialysis phase, traditional risk factors, those inherent to uraemia, and new specific factors related to the ongoing dialysis

modality, work jointly (Bucharles *et al., 2010*). Systemic low-degree chronic inflammation plays a central role in the pathophysiology. Several myocardial alterations, especially those associated with fibrosis and vascular calcifications, occur, justifying many events of sudden death (due to cardiac arrhythmias) and congestive heart failure. Atherosclerotic damage in medium and large calibre arteries accounts for cerebrovascular accident, peripheral vascular disease, and abdominal aorta aneurysm (Bucharles *et al., 2010*).

Figure 1.4. Overview of the pathophysiology and complications in patients with CKD



(Adapted from: Bucharles *et al.,* 2010**. Assessment and management of cardiovascular disease in patients with chronic kidney disease.** Figure 1).

Upon renal injury and at the loss of functioning nephrons, the remaining nephrons lose their ability to autoregulate glomerular pressure, this results in the onset of systemic hypertension to the glomerulus. Subsequent elevations in intraglomerular pressure lead to tubular and glomerular hypertrophy. In addition to these processes, endothelial and podocyte cell injury, as a result of uraemia-associated vasculotoxic and inflammatory damage, induce local inflammation and fibrosis, thus further progressing glomerular decline (Lopez-Novoa *et al.,* 2011).

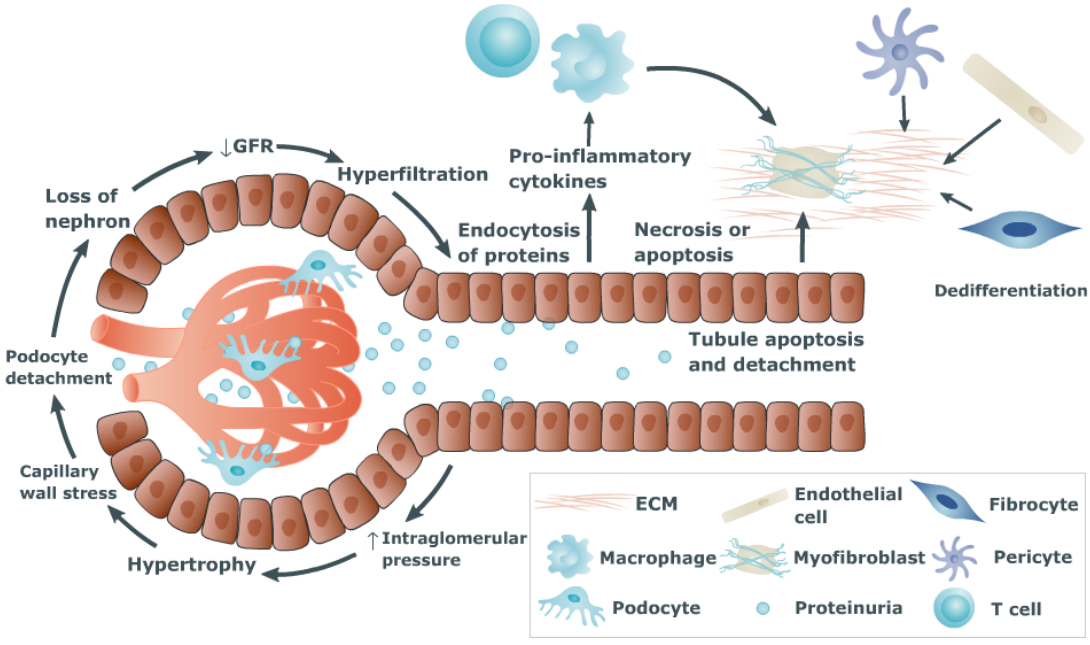
Furthermore, the role of proteinuria (induced by increased intraglomerular pressure), is thought to be regarded as the pathophysiological link between interstitial, tubular and glomerular injury

(Cravedi and Remuzzi, 2013). The extent of proteinuria in glomerular disease correlates with the rate of kidney dysfunction. This is due to the reabsorption of filtered proteins by the tubuloepithelial cells which results in oxidative stress, as well as direct damage to intracellular lysosomal pathways and the release of chemotactic factors. Consequently, these processes lead to the promotion of tubulointerstitial inflammation and fibrosis through the enrolment and activation of macrophages (Fogo, 2007).

In both the tubuli and glomeruli, increased synthesis and reduced degradation of the extracellular matrix ensues as a result of chronic inflammatory processes, with excessive tubulointerstitial accumulation. Subsequently, the formation of tubular atrophy, glomerulosclerosis and tubulointerstitial fibrosis cause further degradation of functioning renal mass, thus formulating an endless cycle of disease progression by increasing intraglomerular pressure and hypertrophy of the residual glomeruli (**figure 1.5**), (Tonolo and Cherchi, 2014).

Lastly, in addition to the preceding factors, angiotensin II is also regarded as playing an important role in the formation of renal artery stenosis (RAS) and is mechanistically involved in most of the disease pathways described above. Produced both locally and systemically in the kidney, angiotensin II exerts several endocrine, autocrine and paracrine effects in the body (Sahay *et al.,* 2012). This potent vasoconstrictor is able to increase intraglomerular pressure by preferentially increasing the efferent arteriolar tone and also enhances intracellular calcium activity in podocytes, causing cytoskeletal changes and altered podocyte function. Furthermore,

Figure 1.5 Development of kidney fibrosis



(Adapted from: Edeling *et al.,* 2016**. Developmental signalling pathways in renal fibrosis..** Figure 1).

angiotensin II causes various increases intubular expression of cytokines, chemokines and growth factors as well as stimulating oxidative stress, resulting in the upregulation of adhesion molecules and chemoattractants (Matsusaka *et al.,* 2010).

CVD in CKD pathophysiology

Hypertension is regarded as a strong risk factor for the development of CKD. Nonetheless, the cause and effect relationship between the two can also be contrasted with regards to CVD; even in the early phases of disease progression, CKD can cause hypertension, which in turn, is likely to increase the risk of CVD in those affected (Gansevoort *et al.,* 2013). This was highlighted by a Japanese study which identified the increased relationship between hypertension and CVD in CKD patients (Kokubo *et al.,* 2009). Moreover, patients with early or advancing CKD have been found to display a higher prevalence of left-ventricular hypertrophy. Research has shown that around 50% of patients with an eGFR below 30ml/min/1.73m2 tend to develop left-ventricular hypertrophy, of which most consist of concentric hypertrophy (Ardhanari *et al.,* 2014)

Aside from hypertension, it is also thought that renal anaemia and increased vascular stiffness may play a pivotal role in the development of left-ventricular hypertrophy (Herzog *et al.,* 2011). Expression of endothelial nitric-oxide synthase is downregulated, suggesting a possible mechanism for coronary endothelial dysfunction in the early stages of CKD. Histologically, left-ventricular hypertrophy in CKD is characterised by myocardial fibrosis which is regarded as impairing contractility. The high prevalence of left-ventricular hypertrophy and the associated risk implications could possibly explain why the chances of sudden cardiac death are increased in those with CKD (Lullo *et al.,* 2015.

Other factors that may contribute towards the raised CVD risk in in CKD patients includes the increased activity of the renin-angiotensin system and sympathetic nerve activity in CKD (Schlaich *et al.,* 2009). The production of superoxide, interlukin 6, and other cytokines is stimulated by angiotensin. In addition, the bioavailability of nitric oxide (which is involved with vascular smooth-muscle contraction and growth), platelet aggregation, and adhesion of leucocytes to the endothelium, are lowered. Combined, all these vasoactive factors cause disturbances to endothelial function (Favero *et al.,* 2014).

One of the most frequent complications of kidney disease is the development of vitamin D due to the impaired activity of the kidney enzyme 1 a-hydroxylase, which converts this precursor to the active hormone. Various studies have demonstrated the associations between vitamin D

deficiency and raised risk of CV events, suggesting that the vitamin D pathway may be involved in the modification of the cardiac structure and function (Bosworth and Boer, 2013).

1.7 Complications

The reduction of GFR enables a varied range of disorders in those with CKD, this includes volume overload, hyperkalaemia, metabolic acidosis and hyperphosphataemia (Wallia *et al.,* 1986). Additionally, hormonal imbalances can lead to the development of anaemia and secondary hyperparathyroidism, accompanied by renal osteodystrophy and systemic dysfunction that develops in the uraemic syndrome, such as anorexia, nausea, fatigue, neuropathy and malnutrition (Tzanakaki *et al.,* 2014).

1.7.1 Sodium and water imbalance

The maintenance of sodium and intravascular volume balance is commonly well preserved until GFR drops below 15 ml/min/1.73m^2. Adaption to changing water intake does, however, begin to diminish in the kidneys as both the maximum dilution and concentration of the urine gradually declines during the course of CKD (hyposthenuria). As patients reach ESRD, urine osmolality remains constant at around 300 mOsm/l (isosthenuria) regardless of actual water volume intake. Consequently, physiological factors other than water intake, urinary dilution and urinary concentration determine the volume of excreted water, thus leading to the development of hypo- and hypernatraemia in CKD patients (Kovesdy, 2012).

1.7.2 Potassium imbalance

Increases in serum potassium levels are common amongst those with renal decline. With a reduction in GFR, potassium excretion is maintained by changes in residual nephrons that increase the efficacy of potassium excretion. As a result of this adaptive response, those with a reduced GFR of less than 15 ml/min/1.73m2 experience extrarenal handling of potassium, especially gastrointestinal excretion, which becomes critical in dispersing an acute potassium load (Hsieh *et al.,* 2011).

1.7.3 Metabolic acidosis

Metabolic acidosis is a common complication of CKD and is a result of the inability of the kidneys to excrete the daily dietary acid load (Kraut and Kurtz, 2005). In the early stages of progressive renal disease, as the overall number of functioning kidney tubules decrease, the tubular functions of the kidneys are diminished thus influencing their ability to produce ammonia and resulting in reduced levels of hydrogen ion excretion and increased bicarbonate excretion. This

excretion of bicarbonate ions leads to the lowering of plasma HCO3-, subsequently causing metabolic acidosis. Additionally, the kidneys also lose the capacity to reabsorb these anions due to a loss in tubular function leading to further anion excretion Kraut and Madias, 2016). In order to maintain the neutrality of electrons, the kidneys retain chloride ions with each bicarbonate ion lost, therefore early CKD is associated with hyperchloraemic metabolic acidosis. Overall, the anion gap remains uninfluenced due to the continual excretion of organic acids by the kidneys (Kovesdy, 2012).

1.7.4 Calcium and phosphorus imbalance

As a result of declining functional nephron mass, the excretion of phosphate becomes disrupted in the kidneys, leading to increases in serum phosphate levels. In order to compensate for the hyperphosphataemia, the parathyroid gland responds by increasing the release of parathyroid hormones (PTH) (Snively and Gutierrez, 2004). Subsequently, increased PTH levels have several effects on the kidneys. Firstly, absorption of calcium is increased, specifically in the ascending limb of Henle. Secondly, PTH also causes an increase in the excretion of phosphorus by blocking reabsorption in the proximal tubule as well as activating 1-hydroxylase which converts vitamin D to its active form: 1, 25 hydroxly vitamin D. Thirdly, PTH also acts on bone by increasing the release of calcium and initiating the activation and proliferation of osteoclasts (Thomas *et al.,* 2008). Once further renal mass is lost and GFR falls below 60 ml/min/1.73m^2, regardless of the compensatory hyperphosphaturia, the eventual onset of hyperphosphataemia is sustained (Murphree and Thelen, 2010).

1.7.5 Anaemia

The onset of anaemia is almost a universal finding in patients with stages 3 to 5 CKD. The anaemia of CKD is commonly normochromic and normocytic in nature, occurring in approximately 50% of patients (Thomas *et al.,* 2008). While CKD-associated anaemia can result from multiple mechanisms (iron, folate, or vitamin B12 deficiency; gastrointestinal bleeding and systemic inflammation), the most common cause of this complication results from decreased erythropoietin synthesis. Erythropoietin, which is essential in the growth and differentiation of red blood cells in the bone marrow, becomes compromised in CKD through the effect of tubular atrophy which generates tubulointerstitial fibrosis and thus resulting in anaemia (Babbit and Lin, 2012).

Additionally, CKD-related anaemia increases the levels of morbidity and mortality from cardiovascular complications (angina, left ventricular hypertrophy and worsening heart failure),

leading to further decline of renal function and the establishment of an endless cycle termed the “cardiorenal anaemia syndrome” (Singh, 2007).

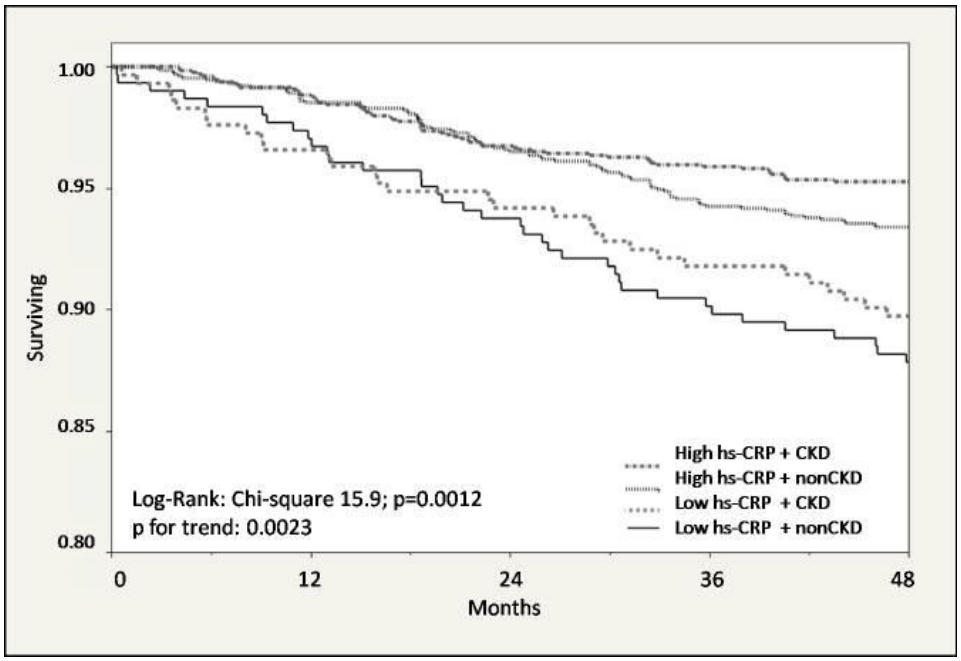
1.8 Early recognition, prevention and treatment of CKD

The ability to recognise early disease onset is one that is highly desirable, primarily due to the interventions that can be established to aid the reduction of CV events or further progression to kidney failure in those affected (James *et al.,* 2010). High prevalence of CKD along with the absence of disease indication, accessibly of laboratory testing, and the availability of treatment measures suggest that screening for CKD would be a valuable and worthwhile possibility in the clinical setting (Jaar *et al.,* 2008). Nonetheless, it must be argued that the action of population-based screening remains controversial (Saunders *et al.,* 2015). Screening for markers such as proteinuria is one that is easy to undertake, efficient in CV risk prediction and may be more effective in defining a reduced GFR than the use of estimated glomerular filtration rate (Sandilands *et al.,* 2013). However, data from a US study which used annual dipstick testing to evaluate proteinuria found that the procedure was not cost effective unless the test was limited to high-risk individuals (e.g. older population, hypertension or diabetes) (Garg *et al.,* 2002). With this in mind, previous literature has highlighted the efficiency of albuminuria testing in young populations, which is one of the most commonly tested marker of CKD in this category. In contrast, a reduced eGFR has been found to occur more frequently in elderly people with the disorder, therefore suggesting that albuminuria and eGFR might have complementary roles in the screening of different age groups, and a combination of both variables could prove to be resourceful in the identification of individuals at high risk of progression to late-stage renal failure (James *et al.,* 2010).

Due to the high associations between inflammation and cardiovascular disease, markers such as high-sensitivity C-reactive protein (hs-CRP) and Interlukin-6 (IL-6) are regarded as powerful predictors of cardiovascular events and mortality in CKD patients (Samak *et al.,* 2002). Furthermore, recent studies indicate that even early onset CKD patients may suffer from inflammation. Using data from the Cerebrovascular Diseases and Dementia in the Community of Ebersberg (INVADE) study, research has shown high levels of hs-CRP to predict the increased risk of vascular events in a CKD population. More specifically, out of the 3,166 participants, 724 individuals (23%) were found to have CKD at baseline. After a 4 year follow up, it was found that precisely 204 (6.4%) individuals experienced a major cardiovascular event. As illustrated in **figure**

1.6, the majority of vascular events occurred in subjects with high hs-CRP/CKD, thus suggesting the inflammatory marker hs-CRP to be of great prognostic value (Jalal *et al.,* 2012).

Figure 1.6. Kaplan-Meier survival curves showing the rate of survival in CKD and non-CKD subjects.



(Adapted from: Jalal *et al.,* 2012**.C-reactive protein as a predictor of cardiovascular events in elderly patients with chronic kidney disease.** Figure 1).

Initial management strategies for CKD involve the identification of reversible conditions (e.g. infection or autoimmune disease) that would be respondent to specific treatment measures and result in the maintenance or improvement of kidney function (Vassalotti *et al.,* 2016). Regardless of the principal cause of renal decline, typical aims for all CKD patients involves the prevention of CV events as well as decreasing the rate of progression of the disease, thereby postponing or averting any related complications.

With regards to treatment measures, hypertension is considered the mainstay of CKD management to aid in slowing renal decline and reduce CV risk. Studies have shown that risk of CKD progression and kidney failure is dramatically increased as blood pressure reaches beyond 130/80mm Hg. Thus, current recommendations set the BP target levels of CKD patients between 125-135/75-85 mm Hg. Although, current guidelines promote a goal of lower than the desired blood pressure in the general population (Drawz and Rosenberg, 2011).

To help reduce further progression of declining renal function, angiotensin-converting-enzyme inhibitors are utilised (Baltatzi *et al.,* 2011). The efficiency of such measures are best seen in proteinuric CKD and are therefore suggested as first-line treatment in this target group. The

collective results of 11 randomised controlled trials revealed the risk of kidney failure or doubling of creatinine concentration serum to be reduced by nearly 40% using an angiotensin-converting-enzyme inhibitor when compared to other forms of antihypertensive drugs in CKD patients (Maschio *et al.,* 1996). Additionally, in those with diabetic nephropathy, angiotensin-converting-enzyme inhibitor exhibits clear benefits in reducing the risk of death, dialysis or transplantation, including a 20% reduced risk of doubled creatinine concentration (Lewis *et al.,* 1993). Findings in a similar population study also showed that targeting glycosylated haemoglobin (HbA1c), to less than 6% reduced the incidence of new cases of microalbuminuria in those with both type 1 and type 2 diabetes (Kuo *et al.,* 2016).

Non-pharmacological treatment measures involved with CKD care include the limitation of dietary sodium intake to an amount of less than 100mmol per day to help manage or prevent hypertension (Wright and Cavanaugh, 2010). The implementation of dietary protein restrictions is also employed in CKD treatment methods with results of meta analyses suggesting a decrease in kidney failure or death with severe reductions or very low protein intake (Bellizzi *et al.,* 2016). However due to the risk of malnutrition and the need for additional monitoring, stark restrictions in protein consumption is generally not valued in patients with CKD (Stark *et al.,* 2011).

Finally, there has been large increments of evidence that suggest obesity as being associated with the development of CKD, as well as the involvement in progression to kidney failure and mortality-related findings (Hall *et al.,* 2014). Although, how much of these effects are facilitated by the complications of diabetes, dyslipidaemia and hypertension remains unclear. Weight gain, which is known to increase the risk of CKD even in patients with normal starting weight, is suggested to be avoided in the setting of renal decline. Whereas those who present as overweight are recommended as losing weight due to known benefits for dyslipidaemia, glycaemic control, and hypertension (Mallamaci and Tripepi, 2013).

Table 1.5 Characteristics of studies evaluating the impact of interventions on a CKD population.

Study, year	Sample size, n	Intervention	Outcome
Howden *et al.,* 2013	90	Lifestyle intervention (Smoking cessation and physical activity	Improved glucose and lipid metabolism. Reduced inflammation with improved endothelial function

Appel *et al.,* 2010	1,094	Blood pressure control	Lowered risk of CKD progression
Modification of diet in renal disease (MDRD) study , 1989	840	Nutritional intervention	Controlled blood pressure. Reduced albuminuria and slowed progression of kidney disease
Bolignano and Zoccali, 2013	2,013	Weight loss intervention	Decreased proteinuria. Increased GFR in obese patients and delayed progression to ESRD in CKD patients

Adapted from: Cramer, 2004. **A systematic review of adherence with medications for diabetes**. Table 1.

1.9 Aims and Objectives

CKD, along with several associated diseases such as diabetes mellitus and hypertension are amongst those that are growing at an unprecedented rate (Levey *et al.,* 2010). In addition, the associated cost implications along with related adverse outcomes of these diseases places a significant financial burden on the healthcare system (Kerr *et al.,* 2012). As previously highlighted, declining renal function, even at the initial stages, is an important independent risk factor for cardiovascular disease, thus several researchers have confirmed that early detection of CKD is essential in preventing CVD morbidity and mortality (Locatelli *et al.,* 2002). Consequently, the implementation of screening programmes for early diagnosis of CKD is imperative, especially in high risk populations due to the silent and unrecognised development of the disease (Kopyt, 2006). By detecting renal failure at the earliest stages of progression, this will allow for more time for the prevention and treatment of primary causes as well as initiation of intervention programmes to aid in slowing the development to end stage renal disease.

The overall objective of this study is to assess and detect the prevalence of CKD risk factors in a general, non-clinical population in order to evaluate the significance of testing in an apparently 'healthy' group of participants and identify those at a higher risk of developing the complications associated with CKD and related diseases.

The specific aims of the study included:

- To study the prevalence of known traditional and novel CKD related risk factors in a non-clinical population.

- Examine the applicability of several predictive equations in the estimation of GFR for the stratification of CKD.
- Investigate the relationships between key risk factors amongst different subgroups of the total study population.
- Provide evidence in the need for further healthcare screening strategies to help identify early onset CKD.

Chapter 2.0
Materials and Methods

2.1 Study Population and method of recruitment

In this study, males and females aged over 18 years and with no existing history of chronic metabolic disease were recruited from the University of Lincoln population. The overall population was split into two age groups comprising of under 30 years and over 30 years of age. Healthy subjects were recruited using several methods; this involved gaining access to the 'Healthy Campus Week' promotion put on for staff by the University. Additional methods of recruitment also involved mass-mailing to heads of Schools offering the opportunity to take part in the study. Younger participants were recruited through undergraduate research projects by the School of Life Sciences. Lastly, from the help of the Human Resources department, further recruitment was conducted via the Global Corporate Challenge event that offered participants the chance to monitor their general health over a 100 day period and witness any changes from a before and after perspective.

To qualify for recruitment, participants' must not be pregnant at the time of testing or have previously been diagnosed with diabetes, cardiovascular problems or kidney disease. Any participants with a clinically diagnosed disorder were informed to consult with the principal investigator. Due to the nature of the bioelectrical impedance device those with any fitted electrical devices, such as pacemakers, were asked not to partake in the study.

All study protocols were approved prior to subject recruitment (see appendix A1) by the University of Lincoln School of Life Sciences Ethics Committee. Written informed consent was obtained from all study participants and secured in a locked cabinet with access only available to the primary researcher and supervisors. Additionally, each individual was assigned a unique participant number to warrant complete confidentiality against all study results.

2.2 Protocol for testing

Testing on participants was carried out at the University of Lincoln Science Building and the Joseph Banks Laboratories. Subjects who met the pre-specified inclusion and exclusion criteria were provided with a brief description of the study (see appendix A2) and invited to attend a screening session. Eligible participants who provided informed consent to the study (see appendix A3) were given a participant information leaflet detailing a more in depth explanation of the study objectives, procedures, risks and benefits and contact information (see appendix A4). On the day of assessment, respondents would arrive to the research facility on the morning or afternoon of their allocated time slot. Prior to testing, individuals were seated and asked to fill out a lifestyle questionnaire (see appendix A5) and, if not yet completed, a study consent

form. Anthropometric and haemodynamic measurements were taken prior to blood sampling. Blood drawn during phlebotomy was for laboratory testing as described in the following sections. Details of materials and equipment used for testing can be seen in **table 2.1** below:

Equipment	Description
Seca 217 stadiometer	Used to measure height (cm)
Seca 770 scales	Used to measure weight (kg)
Seca tape measure	Used to measure waist and hip (cm)
Boso Medicus Uno monitor	Measurement of systolic and diastolic blood pressure (mm Hg) and heart rate
Bodystat 1500MDD	Bioelectrical impedance analysis
Bodystat 0525	Electrode pads (4 per participant)
Folded hospital privacy screen	Used for the privacy needs of participants during measurements

Table 2.1. Equipment used in the body composition phase of the study.

2.3 Anthropometric measurements

All anthropometric and haemodynamic measurements were taken in triplicate to achieve a mean value and increase overall reliability of results.

Height and weight

Body weight measurements were taken to the nearest 0.1kg and height was measured using a fixed stadiometer in all participants after removal of footwear. Body mass index (BMI) was calculated the following way:

$$BMI = \frac{\text{Weight (kg)}}{\text{Height}^2\ (m^2)}$$

Waist and hip measurements

With the participants standing upright behind a curtain, waist circumference (cm) was measured using a tape measure at the point of midway between the costal margin and the iliac crest in the mid-axillary line. Hip circumference (cm) was determined at the widest point around the greater trochanter. Waist-to-hip ratio (WHR) was calculated by:

$$WHR = \frac{\text{waist (cm)}}{\text{Hip (cm)}}$$

Body fat percentage

Participants were required to lie in a rested supine position on the seated bench provided and asked to remove their right sock. Body stat electrodes were then placed along the individuals' extensor retinaculum and extensor digitorum of both the right hand and foot. Age, height, weight and circumference measurements were entered into the machine as well as activity

levels. Participants were again asked if any electrical implants were present on their body. Upon completion of testing, recruits were then able to place any footwear back on.

2.4 Haemodynamic measurements

Blood pressure was obtained at each study visit using a 'Boso Medicus Uno' monitor, a fully automated oscillometric recorder which measures systolic and diastolic pressure as well as heart rate. Participants were seated in a resting supine position and measurements were recorded three times to achieve an average.

2.5 Protocol for phlebotomy

Phlebotomy

All blood was taken by an experienced phlebotomist with adherence to the university's ethical regulations. Due to concerns surrounding the fasting state of participants upon arrival, it was agreed that all participants would arrive in a non-fasting state as to prevent damage or injury to participants' health and avoid unlawful practice. Blood was collected with participants' in a semi-recumbent position using a 4ml Becton Dickinson gold top serum separation tube vacutainer. Samples were then left to clot between 30-60 minutes before being transported to the laboratory for immediate separation and storage; this was necessary due to the rate of glycolysis whereby 7% of glucose is lost from the uncentrifuged sample per hour. All subjects were asked to be seated for a further 5 minutes before leaving the testing facility to monitor for any health implications. All materials necessary for the procedure are highlighted in **table 2.2**.

Equipment	Description
Becton Dickinson gold top serum separation tube vacutainer 4ml	Tube container for participants' blood samples (one per person)
Syringe	-
Blood needle	-
Brady MPB21 label printer	Used to label samples with the participants' unique ID code
Tourniquet	-
Cotton wool buds	-
Plasters	-
Fast aid pre-injection swabs 70% IPA alcohol	Used in the sterilization of participants' arm prior to blood taking

Table 2.2. Equipment used for the phlebotomy procedure.

Sample separation and storage

Sample separation was carried out using a Beckman Coulter Allegra X-15R centrifuge. All samples were centrifuged at 3000 rpm for 5 minutes at 20°C (acceleration and deceleration rate: 9). Pipetted serum layers were placed in a labelled eppendorf tube and stored in the refrigerator at 4°c until required for biochemical analysis. Maximum storage time for this temperature was estimated at 72 hours before all glucose assays would become unascertainable. Additional samples from the same yield were stored at -20°c for future assay. All materials used during this stage are found in **table 2.3** below.

Equipment	Description
Plastic pipettes	Used to aliquot the samples into separate tubes
Eppendorf tubes	Storage of samples until use (three per participant)
Brady MPB21 label printer	Labels to identify participant samples
Virkon solution	Sanitise used or processed materials
Rubber gloves	Prevent contact between researcher and blood samples
Beko LC120W fridge freezer	Used for storage of participants' samples
Beckman Coulter Allegra X-15R centrifuge	Used for the centrifugation of blood tubes

Table 2.3. Equipment used in the sample separation and storage phase.

2.6 Biochemical assays

All assays were performed on the Horiba ABX Pentra 400, an automated clinical chemistry bio analyser which allows for highly accurate photometric measurements of a variety of human biomarkers. Before sample testing, the machine was first calibrated using ABX Pentra Multical, a lyophilized human serum calibrator with chemical additives and materials of biological origin. The assigned values of the calibrator's components were provided in the labelling, ensuring optimal calibration of appropriate Horiba SBX SAS methods on the Pentra 400 analyser. After reconstituting one vial of Multical with 3ml of deionised water, the sample is left to stand at room temperature for no less than 30 minutes. The vial is then slowly agitated, avoiding any formation of foam and then transferred into a sample cup via pipette.

Further calibration of Horiba Pentra N control and P control also took place prior to testing. These were quality control products which consisted of lyophilized human serum with chemical additives and materials of biological origin added as required to obtain given component levels. One vial of each reagent was mixed with 5ml of deionised water and left to stand at room temperature for 30 minutes. Following this, the samples were slowly agitated and the required volume was aliquoted into the sample cups.

Both the calibration and control reagents were placed into the correct position on the instrument sample tray and assigned a location within the machine.

The procedures stated above were also repeated for the fructosamine and hsCRP calibration and control reagents which were then placed into the machine and assigned target values. **Table 2.4** details all necessary materials used in the biochemical testing stage.

Equipment	Catalogue reference number
Deproteinizer CP	A11A01754
Clean-Chem 99 CP	A11A01851
Multi Cal	A11A01652
Fructo Cal	A11A01680
CRP HS Cal	A11A01983
N Control	A11A01653
P Control	A11A01654
Fructo Control N	A11A01681
Fructo Control P	A11A01682
Low CRP Control	A11A01731
Creatinine 120 CP	A11A01933
Glucose PAP CP	A11A01668
Cholesterol CP	A11A01634
Triglycerides CP	A11A01640
Fructosamine	A11A01679
CRP CP	A11A01611
Sample cup (blue)	A11A01766

Table 2.4. Equipment used in the biochemical testing of participants' samples

Parameters that were determined during the study include:

2.6.1 Renal Function

Serum Creatinine

Creatinine measurements are used in the assessment of renal dysfunction. Increased levels of creatinine are evident in renal diseases and insufficiency with reduced glomerular filtration; urinary tract obstruction; decreased renal blood flow including congestive heart failure, shock and dehydration.

Renal function was assessed using the Abx Pentra Creatinine 120 CP reagent. This substance is a diagnostic reagent for quantitative *in vitro* determination of creatinine in human serum and based on a kinetic method using alkaline picrate (Jaffe method).

Estimated glomerular filtration rate (eGFR)

The eGFR (in ml/min/1.73m^2) was calculated using the Modification of Diet in Renal Disease study (MDRD) equation (Levey *et al*., 1999).

eGFR = 186 x [Serum creatinine (µmol/L) / 88.4] $^{-1.154}$ x age (years)$^{-0.203}$ x (0.742 if female) x (1.210 if black)

Additional equations were calculated for comparison to the standardised eGFR equation:

Cockroft-Gault

A commonly used substitute marker for the estimation of creatinine clearance is the Cockcroft-Gault (CG) formula. This method employs the participants' weight and serum creatinine measurements to predict the level of creatinine clearance.

$$eC_{cr} = \frac{(140 - Age) \times Mass\ (kilograms) \times Constant}{Serum\ Creatinine\ (\mu mol/L)}$$

(Where Constant is 1.23 for men and 1.04 for women)

CKD-EPI

The Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation is considered to provide a more accurate prediction of glomerular filtration rate (GFR), along with a lesser bias and greater accuracy (Levey *et al.,* 2009).

GFR = 141 * min(Scr/κ,1)$^{\alpha}$ * max(Scr/κ, 1)$^{-1.209}$ * 0.993^{Age} * 1.018 [if female] * 1.159 [if black]

Scr is serum creatinine (mg/dL), κ is 0.7 for females and 0.9 for males, α is -0.329 for females and -0.411 for males, min indicates the minimum of Scr/κ or 1, and max indicates the maximum of Scr/κ or 1.

2.6.2 Markers of glycaemic control

Glucose

Glucose assessment was carried out using Abx Pentra Glucose PAP CP reagent. Enzymatic determination of glucose was calculated using the Trinder method.

Fructosamine

Abx Pentra fructosamine test is a bi-reagent kit which includes reagents R1 and R2 (NBT reagent/buffer), which was added to the sample. The colorimetric assay was based on the ability of ketoamines to reduce nitrotetrazolium blue (NBT) in an alkaline environment. The speed of

formazan formation was directly proportional to the fructosamine concentration. The reaction speed was measured by automated photometry at 546nm.

2.6.3 Lipid and Lipoproteins

Total cholesterol

Abx Pentra CP test is an enzymatic photometric test which determines the level of cholesterol after enzymatic hydrolysis and oxidation. The colourimetric indicator is quinoneimine which is generated from 4-aminoantipyrine and phenol by hydrogen peroxide under the catalytic action of peroxidase (Trinders reaction).

Triglycerides

In the enzymatic triglyceride method, triglycerides are hydrolysed by lipase to free fatty acids and glycerol. The glycerol is phosphorylated with ATP in the presence of glycerol kinase, and the resulting ADP is coupled to the formation of pyruvate, which is reduced by NADH.

2.6.4 Plasma markers of inflammation and endothelial function

High sensitivity C-reactive protein

C-reactive protein (CRP) is an acute phase protein which tends to increase concentration as a result of the inflammatory process, most notably in response to pneumococcal infections, histolyic disease and a variety of disease states. CRP is used as a marker or general diagnostic indicator of infections and inflammation, in addition to serving as a monitor of patient response to therapy and surgery.

Abx Pentra CRP CP is a latex-enhanced immunoturbidimetric assay developed to accurately measure CRP levels in serum and plasma samples for conventional CRP ranges. When an antigen-antibody reaction occurs between CRP in a sample and anti-CRP antibody which has been sensitised to latex particles, agglutination results. This agglutination is detected as an absorbance change, with the magnitude of the change being proportional to the quantity of CRP in the sample. The actual concentration is then determined by interpolation from a calibration curve prepared from calibrators of known concentration.

2.7 Statistical Analysis

Data entry and statistical analysis was performed on SPSS version 20.0. A two-tailed p value <0.05 was considered statistically significant. All categorical variables were first tested for frequencies to check for response ratios. Additionally, descriptive statistics were carried out on all continuous variables to give the overall characteristics of the data set as well as provide information of the distribution and normality of the scores using the Kolmogorov-Smirnov's test.

Analysis of normality revealed the data to be unevenly distributed and therefore it was decided to test all data as non-parametric. Additionally, due to the sample size of the data in this study it was likely that outliers would exert a dramatic effect upon the correlation coefficient, either resulting in obscurities to the r value or an underestimation of the true relationship. Therefore, it was agreed that a scatterplot would be used to check for any outliers in the data and where possible, the removal or recording of any offending values. With the case of any missing values, SPSS allows descriptives to be run whereby the percentage of values that are missing for each of the variables is highlighted. When dealing with missing data it was agreed that the 'exclude cases pairwise' option would be chosen, as this excludes the cases (persons) only if they are missing the data required for the specific analysis. Importantly, they were still included in any of the analyses where they had the necessary information.

Measures of variability

Standard deviation was used to quantify the level of variation around the mean value of the observations retrieved from the data set. A low standard deviation indicates that the data points tend to be close to the mean value of the set, while a high standard deviation indicates that the data points are spread out over a wider range of values.

Correlation coefficient

To assess the strength and direction between two variables, Spearman's rank correlation coefficient (*r*) was calculated. An *r* value of 1.0 indicated a perfect positive correlation, a correlation of -1.0 indicated a perfect-negative correlation, and a correlation of 0 indicated a random, nonlinear relationship between the two variables. Output variables were interpreted using Cohen's guidelines (1988); where a strong correlation was determined by a result greater than 0.8 and a weak correlation was determined by a result of less than 0.5.

To indicate the percentage of shared variance between the two variables, 'coefficient of determination' was calculated. This test allowed for the proportion of fluctuation of one variable that is predictable from the other variable. The coefficient of determination was calculated by

squaring the *r* value. This result was expressed further by multiplying by 100 and giving the 'percentage of variance'.

Comparison of means

Parametric tests

An independent samples t-test was carried out on the data set to test for differences between two independent groups on a continuous measure. This test allowed for the statistical testing of probability that the two sets of scores came from the same population.

Non-parametric tests

A Friedman Test was carried out for one-way repeated measures analysis of variance by ranks. After establishing a statistically significant difference amongst the three ranks, post-hoc testing was carried out including the Wilcoxon Signed Rank Test. Due to the involvement of two tests, a revised Bonferonni adjusted value was applied giving an alpha level of determined statistical significance of less than .025.

Chapter 3.0
Results

3.1 Socio-demographic and clinical characteristics of the study population

Overall, a total of 186 participants were recruited for this study. Amongst these individuals, 64% were female with a mean age of 31 years. In comparison, 36% of the total population was male with a mean age of 27 years. **Table 3.1** displays the main sociodemographic and clinical characteristics of the study population according to gender. Analysis of gender differences revealed that females had a significantly higher body fat percentage (p = <0.001) and heart rate (p = .006). In contrast, males were found to have a higher systolic blood pressure (p = <0.001) and waist to hip ratio (p = <0.001).

Table 3.1 Socio-demographic and clinical characteristics of the study population according to gender.

Clinical status	**Females**	**Males**	**Sig.**
	Mean [SD] (%)	Mean [SD] (%)	
N	119 (64%)	67 (36%)	-
Age (years)	31 [12.8]	27 [11.2]	.031*****
Ethnicity			
Caucasian	(90.8%)	(86.6%)	.445
Black	(2.5%)	(4.5%)	"
Asian	(6.7%)	(9.0%)	"
BMI (kg/m^2)	24.9 [6.2]	24.8 [4.0]	.864
Systolic blood pressure (mmHg)	123 [13.2]	136 [13.0]	<0.001*****
Diastolic blood pressure (mmHg)	79 [10.3]	80 [10.6]	.548
Heart rate (bpm)	76 [11.3]	70 [13.4]	.006*****
Body fat%	31 [9.2]	17.6 [6.2]	<0.001*****
Waist: hip	0.79 [.062]	0.90 [.086]	<0.001*****
Family history of			
Heart disease	(21.8%)	(20.9%)	0.989
Type 1 diabetes	(4.2%)	(6%)	.548
Type 2 diabetes	(17.6%)	(22.4%)	.355
CKD	(3.4%)	(10.4%)	.138
Stroke	(10.9%)	(7.5%)	.495

Anaemia	(6.7%)	(4.5%)	.577
Hypertension	(33.6%)	(31.3%)	.893
Smoker	(10.1%)	(17.9%)	.173

N, number of subjects; BMI, body mass index; bpm, beats per minute; mmHg, millimetre of mercury.
***** = Statistically significant result

3.2 Laboratory parameters

Results from the analysis of biochemical components revealed two key significant differences between the gender groups. This includes a higher level of triglycerides in males (Mean = 1.2, SD = 0.58, P = 0.004), and a higher level of creatinine in males (Mean = 81, SD = 15.8, P = <0.001). However, although significant, these measurements were within the normal range for both genders (**Table 3.2**).

Table 3.2 Laboratory parameters in the recruited participant population.

Laboratory parameter	**Females** Mean [SD]	**Males** Mean [SD]	**Sig.**
Lipids			
Total cholesterol (mmol/l)	4.24 [.89]	4.1 [.86]	.274
Triglycerides (mmol/l)	0.98 [.47]	1.2 [.58]	.004*****
Diabetes mellitus			
Glucose (mmol/l)	4.49 [.90]	4.69 [1.1]	.181
Fructosamine (µmol/l)	221 [29.6]	227 [40.0]	.224
Kidney			
Serum creatinine (µmol/l)	63 [13.3]	81 [15.8]	<0.001*****
eGFR (mls/min/1.73m^2)	111 [31.0]	113 [26.0]	.661
Inflammation			
High sensitive CRP (mg/l)	1.39 [1.9]	1.4 [2.1]	.976

CRP, C-reactive protein; eGFR, estimated glomerular filtration rate (calculated using the modification of diet in renal disease formula). N = 119 for females and 67 for males.

3.3 Age comparisons

Overall, age comparisons of the separate genders indicated several significant differences between the two groups. Results from the male gender category are displayed in **table 3.3**. This shows a slight increase in several parameters in the over 30's group which includes; BMI (mean = 24 vs 26 over 30's, p=0.114), diastolic blood pressure (mean = 77 vs 87, p=0.001), waist to hip ratio (median = 0.87 vs 0.93, p=0.016), and body fat percentage (mean = 16 vs 22, p=0.001). In contrast, several parameters also revealed increases in the under 30's group, this includes; heart rate (mean = 72 under 30's vs 65, p=0.042), glucose (mean = 4.8 vs 4.4, p=0.216), and serum creatinine (mean = 84.1 vs 71, p=0.004). Likewise, the female gender group also revealed similar results with the over 30's category showing higher readings in; body mass index (mean = 24 vs 27 over 30's, p=0.004), diastolic blood pressure (mean = 76 vs 84, p=<0.001), and body fat percentage (mean = 28 vs 35, p=<0.001). Similarly, increases were also observed in the under 30's group, more specifically in the creatinine readings which showed a mean result of 65ml/min/1.73m^2 in comparison to the over 30's which was observed at a mean of 59ml/min/1.73m^2 (**table 3.4**).

Upon further analysis, several parameters showed results outside the boundaries of a healthy range. This included BMI which appeared to be higher in males and females aged over 30 (mean in males = 25, SD =7.2, mean in females = 26, SD =3.7), as well as body fat percentage which was substantially higher in females aged over 30 years (mean = 35, SD = 9.3, p = <0.001).

Table 3.3 Age comparisons between the male study-population.

Females

Parameters	**Under 30 years** Mean [SD]	**Over 30 years** Mean [SD]	**Sig.**
N	71	48	-
BMI (kg/m^2)	24 [5.0]	27 [7.2]	.004*****
Systolic blood pressure (mmHg)	121 [12.4]	126 [14.0]	.037*
Diastolic blood pressure (mmHg)	76 [9.4]	84 [9.8]	<0.001*****
Heart rate (bpm)	78 [11.3]	72 [10.6]	.006*****
Body fat%	28 [7.8]	35 [9.3]	<0.001*****
Waist: hip	0.8 [0.07]	0.79 [0.06]	.443
Total cholesterol (mmol/l)	4.2 [0.82]	4.3 [0.92]	.397

Triglycerides (mmol/l)	0.98 [0.47]	0.97 [0.48]	.885
Glucose (mmol/l)	4.6 [0.93]	4.3 [0.83]	.036*
Fructosamine (µmol/l)	224 [29.3]	217 [29.9]	.203
Serum creatinine (µmol/l)	65 [12.9]	59 [13.3]	.018*
eGFR (mls/min/1.73m^2)	112 [29.3]	109 [33.6]	.643
High sensitive CRP (mg/l)	1.55 [2.1]	1.15 [1.6]	.273

BMI, body mass index; bpm, beats per minute; mmHg, millimetre of mercury; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate (calculated using the modification of diet in renal disease formula).

Table 3.4 Age comparisons between the female study-population.

Males

Parameters	**Under 30 years** Median [percentile]	**Over 30 years** Median [percentile]	**Sig.**
N	50	17	-
BMI (kg/m^2)	24 [4.1]	26 [3.7]	.114
Systolic blood pressure (mmHg)	135 [12.8]	139 [13.9]	.388
Diastolic blood pressure (mmHg)	77 [10.2]	87 [8.6]	.001*
Heart rate (bpm)	72 [13.8]	65 [10.8]	.042*
Body fat%	16 [5.9]	22 [5.2]	.001*
Waist: hip	0.89 [.09]	0.93 [.07]	.056
Total cholesterol (mmol/l)	4.0 [.86]	4.3 [.85]	.330
Triglycerides (mmol/l)	1.2 [.55]	1.1 [.67]	.574
Glucose (mmol/l)	4.8 [.99]	4.4 [1.2]	.216
Fructosamine (µmol/l)	232 [41.3]	212 [32.3]	.076
Serum Creatinine (µmol/l)	84.1 [15.1]	71 [14.2]	.004*
eGFR (mls/min/1.73m^2)	111 [24.8]	116 [30.0]	.504
High sensitive CRP (mg/l)	1.47 [2.24]	1.19 [1.5]	.630

BMI, body mass index; bpm, beats per minute; mmHg, millimetre of mercury; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate (calculated using the modification of diet in renal disease formula).

3.4 Analysis of kidney function

To ascertain the associations between kidney function and the measured parameters, a Mann-Whitney U test was performed on the split-gender population. Results from the analyses revealed similar outcomes with several biochemical parameters becoming significantly correlated with kidney function. In the male population, serum glucose (r = -0.555, p = <0.001), cholesterol (r = -0.475, p = <0.001), triglycerides (r = -0.436, p = <0.001), and fructosamine (r = -0.427 p = <0.001) results revealed a strong negative correlation with eGFR. These results imply that as eGFR decreases, the biochemical parameters increase (**table 3.5**). Similarly, **table 3.6** shows results from the female population which indicates a strong negative correlation between the same biochemical parameters and eGFR as shown in the male gender group. Results include; serum glucose (r = -0.541, p = <0.001), cholesterol (r = -0.618, p = <0.001), triglycerides (r = -0.422, p = <0.001), and fructosamine (r = -0.488 p = <0.001). Additionally, significance was also observed between waist to hip ratio and eGFR (r = -0.354, p = <0.001).

Table 3.5 Spearman correlation coefficients between eGFR and the correlated variables in the male participant population

Males

Variable	eGFR		
	Correlation coefficient (*r*)	**Sig.**	**Percentage variance (%)**
Age	-.009	.944	.008
Waist:hip	-.212	.085	4.5
BMI (kg/m^2)	.015	.906	.023
Systolic (mmHg)	-.148	.233	2.2
Diastolic (mmHg)	.099	.427	0.99
Heart rate (bpm)	-.051	.680	0.26
Body fat %	.004	.973	.002
Glucose (mmol/l)	-.555	<0.001*	30.1
Cholesterol (mmol/l)	-.475	<0.001*	22.6
Triglycerides (mmol/l)	-.436	<0.001*	19
Fructosamine (µmol/l)	-.427	<0.001*	18.2
hsCRP (mg/l)	.061	.624	0.37

BMI, body mass index; bpm, beats per minute; mmHg, millimetre of mercury; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate (given as ml/min/1.73m^2).

Table 3.6 Spearman correlation coefficients between eGFR and the correlated variables in the female participant population.

Females

Variable	eGFR		
	Correlation coefficient (*r*)	**Sig.**	**Percentage Variance (%)**
Age	-.045	.628	0.2
Waist:hip	-.354	<0.001*	12.5
BMI (kg/m^2)	.113	.220	1.3
Systolic (mmHg)	-.136	.141	1.9
Diastolic (mmHg)	.081	.379	0.66
Heart rate (bpm)	-.165	.072	2.7
Body fat %	-.013	.891	0.02
Glucose (mmol/l)	-.541	<0.001*	29.3
Cholesterol (mmol/l)	-.618	<0.001*	38.2
Triglycerides (mmol/l)	-.422	<0.001*	17.9
Fructosamine (µmol/l)	-.488	<0.001*	23.8
hsCRP (mg/l)	.037	.693	0.14

BMI, body mass index; bpm, beats per minute; mmHg, millimetre of mercury; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate (given as ml/min/1.73m^2).

Post hoc testing revealed a strong level of association between eGFR and glucose, with a percentage variance level of 30.1% in males and 29.3% in females. The relationship between glucose and eGFR is illustrated in **figure 3.1**, this shows a strong negative correlation between the two variables. Likewise, fructosamine indicated a strong level of correlation against eGFR (**figure 3.2**), with a percentage variance of 23.8% in the female population. However, a more scattered distribution of results is evident in the males which is reflected by the decreased percentage variance of 18.2%.

Figure 3.1a Correlation between eGFR and serum glucose levels in males and females.

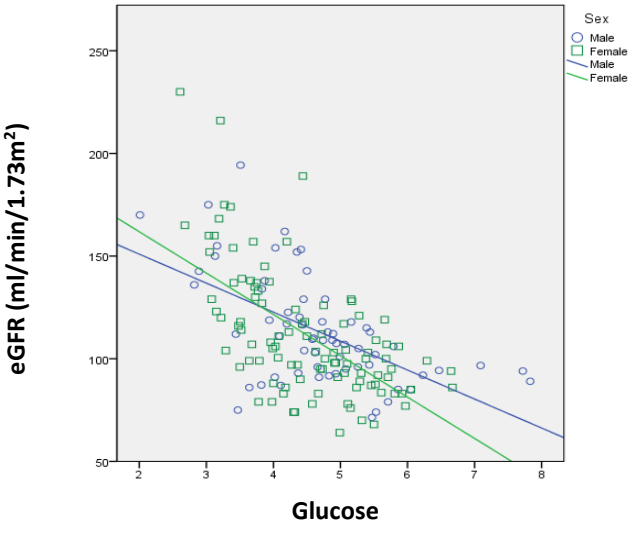


Figure 3.1b Correlation between eGFR and serum fructosamine levels in males and females.

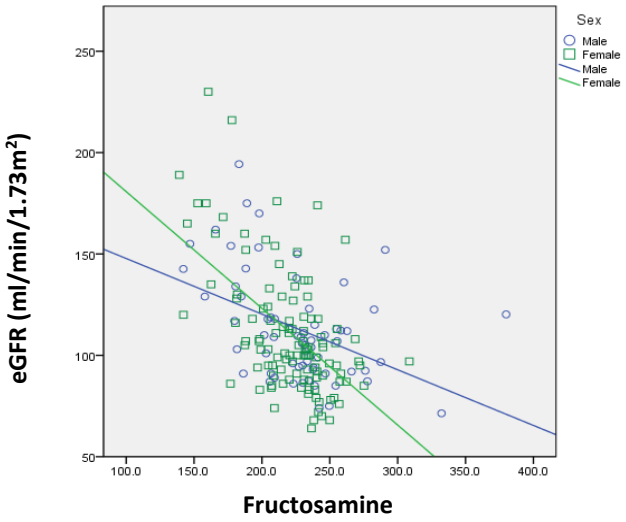


Figure 3.1c Correlation between eGFR and serum cholesterol levels in males and females.

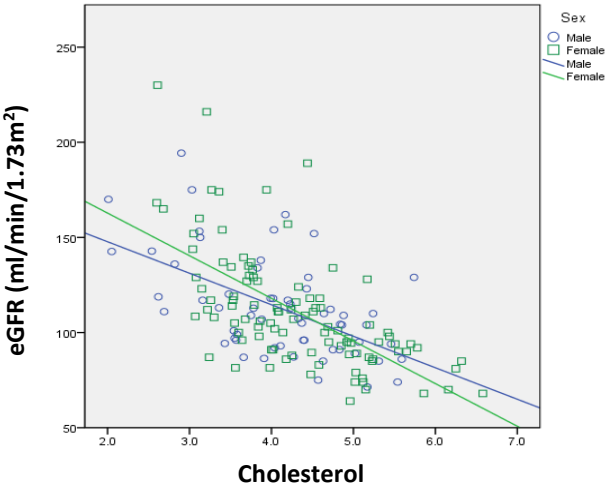


Figure 3.1d Correlation between eGFR and serum triglyceride levels in males and females.

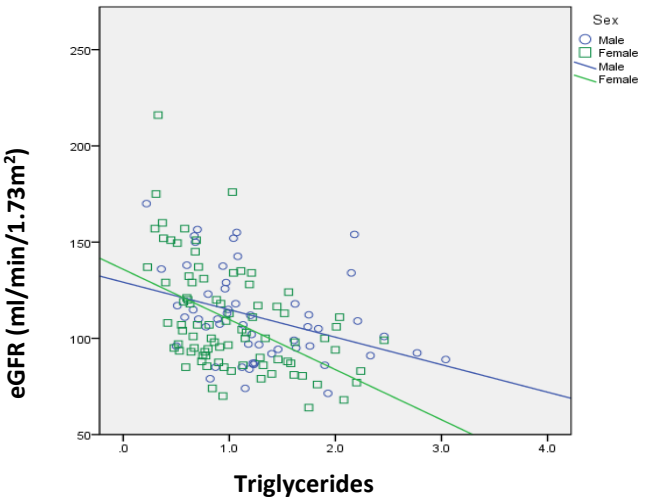


Figure 3.1. Results from the Spearman rho tests showing scatterplot graphs between eGFR and several biochemical parameters including glucose, fructosamine, cholesterol

Analysis of the lipid-based parameters showed high significance between eGFR. The percentage variance of cholesterol in female participants was particularly high at 38.2% which is illustrated by a strong negative correlation in **figure 3.3**. Although lower, males showed a significant correlation between cholesterol and eGFR with a percentage variance figure of 22.6%. In comparison to cholesterol, triglycerides were established to show a much lesser relationship between eGFR in both males (% var = 19%) and females (% var = 17.9%). Nonetheless, a slight negative correlation can still be identified, therefore indicating a causal relationship between the two factors (**figure 3.4**).

3.5 Comparisons between kidney equations

To evaluate the contrast between several creatinine-based GFR estimation equations, a Friedman test was carried out. Investigations revealed a statistically significant difference between the three equations (MDRD, Cockcroft-Gault, CKD-EPI), (n = 186, p = <0.001). Further inspection of the median values also showed Cockcroft-Gault as having the highest estimation of kidney function (median = 125ml/min/1.73m^2, percentile = 100-161), followed by CKD-EPI (median = 111ml/min/1.73m^2, percentile = 97-123),

and MDRD (median = 105ml/min/1.73m^2, percentile = 91-123). The differences between the three variables are illustrated in figure **3.5**.

Although the results indicated a significant difference between the equations, this does not describe where the differences lie. To explore the relationship further, post-hoc testing was carried out using individual Wilcoxon Signed Rank Tests. Additionally, to control for type 1 errors, a Bonferonni adjusted alpha value was calculated (p = 0.017). Results from the post-hoc tests are displayed in **table 3.7**. Findings revealed a statistically significant difference between Cockcroft-Gault and MDRD (p = <0.001) with a medium to large effect size (r = 0.47), as well as Cockcroft-Gault and CKD-EPI (p = <0.001, r = 0.43), however due to the adjusted alpha value of 0.017, MDRD and CKD-EPI were not reported as statistically different from one another (p = 0.044) and only showed a small effect size between the two equations (r = 0.11). Upon further inspection of the mean rank values, Cockcroft-Gault gave the highest level of results with a mean rank of 2.51. In contrast, the CKD-EPI equation showed an intermediate set of results with a rank value of 1.95. Lastly, the MDRD equation revealed a score towards the lower end of the rankings at 1.54.

Figure 3.2 Error bar graph showing comparisons between the MDRD, Cockcroft-Gault and CKD-EPI equations in the total study population.

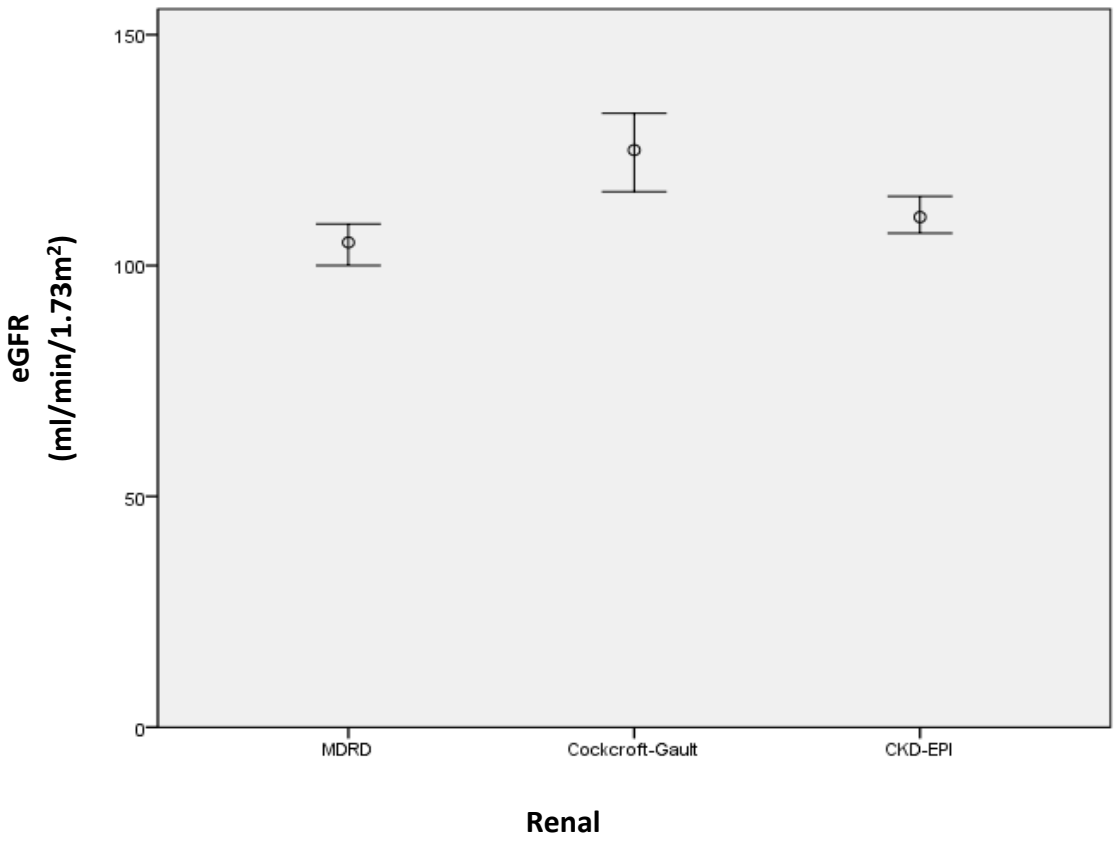


Table 3.7 Wilcoxon Signed Rank Test of the three kidney function equations

Sample 1 – sample 2	***Z***	***sig.***	***r***
Cockcroft-Gault – MDRD	-9.001	<0.001	0.47
CKD-EPI – MDRD	-2.017	0.044	0.11
CKD-EPI – Cockcroft-Gault	-8.360	<0.001	0.43

*Bonferonni adjusted alpha value = 0.017

Chapter 4.0
Discussion

4.1 Study Review

The diagnosis of chronic kidney disease is now relatively straightforward. However, the proportion of patients with end-stage renal disease who are assessed for the first time by nephrologists prior to initiation of dialysis is still unacceptable. Timely diagnosis and immediate referral are significant steps in CKD management. Consequently, this would allow for the implementation of preventative measures which delay or even halt the progression of CKD as well as decrease initial morbidity and mortality (Bastos and Kirsztakn, 2011). In this multifactorial research study of a mixed-aged, non-clinical population, it was found that several of the key predictors including age, BMI and blood pressure, showed a weak relationship between a reduced kidney function. However, anaylsis of several traditional biochemical parameters including serum glucose, fructosamine, cholesterol and triglycerides revealed a strong negative correlation between eGFR, meaning, as kidney function decreased, these parameters increased.

As previously stated, the initial aims of this study was to investigate the prevalence of CKD risk markers in a non-clinical, general population and to determine the relationships between several key parameters such as traditional and novel risk markers, whilst also establishing whether these relationships vary in different groups. It was therefore hypothesised that there would be a significant difference between gender and age groups with regards to kidney function, with high-risk individuals/populations presenting an increased level of well-known and established risk markers when compared to the low risk groups.

4.2 Findings and implications

4.2.1 Gender comparisons

To evaluate the relationships between the study parameters, a gender comparison assessment was carried out. This allowed for the investigation of gender-influence and identify whether males and females should be separated prior to further examination of the study results. As expected, a comparison between the sexes of the participants (table 3.1) revealed a strong diversity in several body composition measurements. This included a significant difference in the haemodynamic results which showed a higher median value of systolic blood pressure in the male population. Reasons for this are supported by Dubey *et al.,* and Reckelhoff who highlighted the key differences in male and female blood pressure due to hormonal and physiological differences (Dubey *et al.,* 2002. Reckelhoff, 2001). Their findings revealed a higher proportion of pre-menopausal females with a lower arterial blood pressure than age-matched men. Similar

findings were also observed by Khoury *et al.,* who's study on a Danish population of 352 normotensive adults, showed men were found to have a higher 24-hour mean blood pressure, by approximately 6 to 10 mm Hg, than did women up till the age of 70 to 79 years (Khoury *et al.,* 1992). As previously discussed, the strong relationship between CKD and hypertension has been shown prospectively in men. A study investigating nearly 12,000 hypertensive male veterans found that the relative risk of end-stage renal disease for pretreatment systolic BP of 165-180 mmHg was 2.8 times higher and 7.6 times higher in those with a systolic BP greater than 180 mmHg, when compared to a systolic BP of less than 140 mmHg (Perry *et al.,* 1995).

Amongst the differences displayed between the two gender groups, body fat percentage was the most highly foreseen. Freedman *et al.,* 1990 displayed these differences in genders through the measurement of proportions in body fat. As with their findings, the results from this study showed men to have a lower overall body fat percentage when compared to women. However, these findings are not unexpected as it has been well established that women generally have a higher percentage of body fat than men (Vella and Kravitz, 2002). Fat in normal women tends to represent between 21-33% of body weight, this is reduced in men however, where fat only represents 10-22% of body weight (Blaak, 2001).

Biochemical parameters

It has long been recognised that serum lipids may contribute towards CKD progression, with previous studies reporting both hypertriglyceridaemia and low high density lipoprotein (HDL) cholesterol as independent risk factors for renal failure (Kurella *et al.,* 2005). As highlighted in the beginning of this thesis, dyslipidaemia and abnormalities in lipid metabolism are known to contribute towards glomerulosclerosis and are common in renal disease (Kumar *et al.,* 2014). Results of the present study partially confirmed the differences in lipid profile between both sexes as men were found to have a higher triglyceride value (1.07 mmol/l) when compared to women (0.86 mmol/l). Reasons for the higher values in males are suggested by the limited number of male participants that took part in the study (36%), therefore a more equal ratio of men and women may have reduced the overall outcome and produced more reliable readings. Nonetheless, higher triglyceride levels are found to be more common in males as shown in a study by Seidell and colleagues who found men with higher triglyceride levels were also at a higher risk of CKD (Siedell *et al.,* 1991). When compared to the female population, no associations were present between any of the serum lipids and CKD.

As previously outlined, the accurate assessment of kidney function among CKD patients is essential for interventional and diagnostic aims. Due to the many disadvantages of using

creatinine clearance and other markers, it is vital that predictive equations are applied to estimate renal function (Coresh and Stevens, 2006). The importance in the role of renal equations can be seen from our results which indicates a higher level of creatinine in the male population (82 µmol/L) when compared to the females (66 µmol/L). Despite this, present knowledge tells us that the cause of higher readings in men is due to the increased muscle mass and therefore greater excretion of creatinine in the body (Baxmann *et al.,* 2008). This is reflected in the estimated glomerular filtration rates of the study population which takes into account the age, race and gender of our participants, thus producing results which show a much closer relation in average kidney function between the two sexes (Males = 109 ml/min/1.73m^2 and females = 103 ml/min/1.73m^2).

Overall, the present findings are indicative of a significant difference in both the body and biochemical results, thus providing the necessary evidence to split the groups by gender when investigating further readings. The splitting of gender groups allows for a more accurate set of results which are less misinterpreted simply due to the physiological differences of the population.

4.2.2 Age comparisons

Findings from the independent samples t-test on age comparisons (table 3.3 and 3.4) revealed a strong difference between body fat percentage and body mass index. The findings echoed that of previously defined literature, whereby increases in body fat and BMI are found with increases in age (Kalyani *et al.,* 2014). One study investigating the interactions between age, BMI and body fat found BMI to progressively increase with age in women and plateaued between 40 and 70 years in men (Meeuwsen *et al.,* 2010). More interestingly, an earlier publication examining the effect of aging on body fat found there to be no further significant increases in body fat after the age of 40, reasons for which are due to the unaccountability of an increased BMI that commonly occurs at middle age due to decreases in organ metabolic rates and resting metabolic potential (Silver *et al.,* 1993). The abundant increase in such factors raises the question as to whether stricter assessment criteria should be implemented in these age-related populations and investigated more vigorously, on the accountability that increased BMI and body fat results in a higher risk of CVD and CKD (Stenvinkel *et al.,* 2013).

Assessment of haemodynamic function in the differential age groups revealed an increase in the diastolic blood pressure of the over 30's population when compared to the under 30's (table 3.2

and 3.3). It is thought that age-related increases in blood pressure are a common occurrence and one that is monitored closely due to the high associations between hypertension and development of CVD risk (Benetos *et al.,* 2000). Although increases were reported on diastolic bp, there were no significant differences observed in the systolic measurements between the two age groups. These findings prove contradictory to previous reports however which indicate a continuous rise in systolic bp with advancing age while a stabilization or decline is observed in diastolic bp (Tin *et al.,* 2002). As an increase in diastolic bp is evident in both sexes, one would deviate from gender influenced results and suggest that these differences may have been observed due to an imbalance in the age ratios of the study population.

Evaluation of serum creatinine between the age groups revealed a decreasing level of creatinine with age. One reason to support this finding is the associations between lower than normal muscle mass and reductions in dietary protein intake, both which are somewhat common with an aging population and can lead to falsely depressed serum creatinine levels (Odden *et al.,* 2009). Studies have suggested that the use of serum creatinine as an indirect filtration marker is limited by its biological and environmental variability ((Musch *et al.,* 2006). Contrary to these findings however, some studies have reported conflicting evidence which states that serum creatinine steadily increases with age (Tiao *et al.,* 2002). A study by Pottel *et al* in 2008, discovered a serum creatinine-age pattern which was constant between the ages of 20 and 70 years. Nonetheless, it has been highlighted that covering such a broad age-range in a small sample size does not allow for the accurate determination of age-specific reference intervals (Pottel *et al.,* 2008).

4.2.3 Analysis of kidney function

Body composition

In relation to the anthropometric measurements (table 3.5 and 3.6) , results from the Mann Whitney U test revealed no significant relationships between kidney function and the body composition readings, aside from wait to hip ratio in women (sig = <0.001) . These finding are surprising due to the well documented outcomes of associations between BMI, blood pressure and waist circumference, which are known to be independently associated with CKD (Navaneethan *et al.,* 2012 and Burton *et al.,* 2011). One study investigating the influence of anthropometric measurements on renal function found the increases of anthropometric measurements to negatively affect kidney function, with BMI producing a more prominent

outcome. For this reason, it is suggested that individuals with increased anthropometric measurements should be monitored closely in terms of renal functions additional to cardiovascular risk factors (Koc *et al.,* 2006). As with our results, the outcome of increased associations between WHR and eGFR only in females, may be explained by the lesser number of male participants who still show a weak but insignificant correlation between the parameters.

Markers of diabetes

Concerning the biochemical measurements, the diabetic parameters including serum glucose and fructosamine showed a strong negative correlation with kidney function. As shown in figures 3.1 and 3.2, eGFR decreases as the measurements increase. Supporting evidence tells us that diabetic nephropathy is one of the most common outcomes from poor glycaemic control, resulting in reduced kidney function and even end-stage renal failure (Schrijvers *et al.,* 2011). As previously outlined, hyperglycaemia causes capillary vasodilation as well as high glomerular hydraulic pressure, therefore leading to glomerulosclerosis and hypertension which causes further damage to the kidneys (Marre *et al.,* 1999). Interestingly, an investigation into the benefits of glucose lowering therapy found that efficient glycaemic control reduces hyperfiltration and hence improved kidney function overall (Meeme *et al.,* 2009). Considering that blood glucose levels are easily affected by caloric intake (Gannon and Nuttall, 2004), this study did not require fasting by participants prior to blood sampling. Subsequently, glucose may have been a poor marker at investigating kidney function. Despite this however, studies have highlighted the question around the need of an 8-hour fasting duration. Moebus *et al*, discovered higher mean blood glucose levels only in the first 3 hours after a caloric intake (Moebus et al., 2011). This observation was also in line with previous findings which stated that in nondiabetic individuals, blood glucose levels return to preprandial levels within 2-3 hours (Sindoni, 1946 and American Diabetes Association, 2001).

Likewise, fructosamine levels were increased in those with a lesser renal function. As fructosamine levels are representative of glycaemic control over a two to three week period, this further cemented our findings and was consistent with previous reports (Vos *et al.,* 2011). As testing was not conducted over a prolonged period, this marker was effective in portraying the glycaemic nature of our study population over a two to three week period. Although, it was debated whether more accurate assessment of glycaemic control may have been highlighted better using either glycated haemoglobin (HbA1c) or glycated albumin (GA) markers (Vos *et al.,* 2012)

Lipids

Analysis of total serum cholesterol revealed a highly correlated relationship between kidney function with a percentage variance of 22.6% in males and 38.2% in females (Table 3.1 and 3.2). As to whether this relationship was caused by an increase in HDL or LDL cholesterol, it is unknown. The inability to ascertain and quantify between the two components was due to the limitations of the assay, however as discussed in chapter 1, it is well known that patients with impaired renal function exhibit significant alterations in lipoprotein metabolism, which in their most advanced form may result in the development of severe dyslipidaemia (Tsimihodimos *et al.,* 2011). Moreover, several epidemiological studies have demonstrated HDL-cholesterol to be a negative risk factor for atherosclerosis (Anavekar *et al.,* 2004. Weiner *et al.,* 2004). As atherosclerosis shares a well-established relationship with CKD (Luczak *et al.,* 2011), it is thus unsurprising that patients with CKD are found to exhibit reduced levels of HDL-cholesterol when compared to those with normal renal function (Olechnowicz-Tietz *et al.,* 2013). Nonetheless, contradictory reports have demonstrated a null association of HDL cholesterol with ESRD in a CKD population, thus postulating the hypothesis that HDL particles are rendered dysfunctional in some manner in CKD (Zewinger *et al.,* 2014). Dissecting the complex interplay of HDL cholesterol, as well as the size of particles, composition and function in CKD is one that requires further investigation using strong epidemiology designs (Boer and Brunzell, 2014).

In CKD patients, hypertriglyceridaemia proves to be one of the most common quantitative lipid abnormalities (Attman *et al.,* 2009). Studies investigating this relationship have demonstrated an increasing concentration of triglyceride-rich lipoproteins (VLDL, chylomicrons and their remnants) in the early stages of CKD (Tsimihodimos *et al.,* 2011). In this study, triglycerides shared a similar result to that of cholesterol; with increases in triglycerides accompanied by a decrease in kidney function levels (figure 3.4). These findings are consistent with previous studies which highlight impaired renal function as exhibiting increased concentrations of triglycerides (Charlesworth *et al.,* 2005). Interestingly however, some reports have identified the similar affects taking place, even in those with a serum creatinine within the normal limits (Fliser *et al.,* 1998).This evidence thus suggests the need for further investigations into the role of fats in CKD, which may be key to early identification of forthcoming reductions in renal function.

Inflammation

Analysis of hsCRP results showed no significant relationship with kidney function. In comparison, previous studies have showed inconsistent findings in the relationship between inflammation and progression of kidney function in a general population (Stuvelling *et al.,* 2003 and Shankar *et al.,* 2011). The result of a significant association between the parameters was identified in the

PREVEND study (Prevention of Renal and Vascular End stage Disease) which identified a strong relationship between hsCRP and lower eGFR in a non-diabetic population (Fox *et al.,* 2010). However, contrastingly, Shankar *et al.* (2011), Indicated that elevated levels of hsCRP were not associated with progression to CKD in a population-based cohort. Evidently, it is clear that this relationship is not yet fully understood. The potential mechanisms which are responsible for the associations between inflammation and renal function declines was explored by Nand *et al.,* (2009) who found that raised levels of hsCRP were closely associated with poor outcomes due to interplay of a number of mechanisms such as lower haemoglobin, high incidence of cardiovascular disease, poor nutritional outcomes, and lower body mass index. In acute inflammation such as infection, CRP levels rise up to 50,000 times fold. It is therefore suggested that such possible outcomes may be explored further through a white cell count to identify those that are displaying such levels.

4.2.4 Kidney equation comparisons

As discussed previously, the current diagnosis, management, and evaluation of CKD routinely relies on estimates of glomerular filtration rate. In our study, we derived the eGFR of the participants using the Modification of Diet in Renal Disease (MDRD) study equation, based on age, race, gender and serum creatinine levels of the assessed individuals (MDRD). The prognostic value of this GFR estimation has been validated in various studies and populations (Levey *et al.,* 2009). However to compare the standard of validity, we tested the abilities of the MDRD equation against several others, including the Cockcroft-Gault (CG) study equation and more recent, Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula.

As expected, we found that the CKD-EPI equation provided a more accurate assessment of renal function within our study population, this finding is non-surprising as it has been well established by recent studies that the CKD-EPI equation, based on the same demographic and laboratory variables as the MDRD study equation, has been shown to represent a far more accurate estimation than MDRD, even more so at higher values of eGFR (Levey *et al.,* 2009 and Stevens *et al.,* 2010).

Analysis of figure 3.5 details the variations between the equations, with CG showing a much higher level of predicted kidney function and MDRD indicating an underestimation of function. Previous reports have commented on the limitations of CG and MDRD, primarily due to the fact they were developed on data from patients with reduced renal function. In comparison, the

CKD-EPI equation was developed to eliminate the weak points of the former, with the data set of the formula taken from many participants with normal GFR in the development process (Levey *et al.,* 2009).

Overall, it is fair to say that our results are consistent with other published investigations. A study by Matsushita *et al.,* (2012) on more than 1 million participants spanning over 40 countries, discovered approximately one-fourth of participants who were reclassified to a higher eGFR category using the CKD-EPI equation in comparison with the MDRD study equation. Effectively, these results lowered the prevalence of CKD in 24.4% of the general population cohort. In addition, their findings also revealed a more accurate estimation clinical risk in both Asians and Blacks, even though reclassification is much less common in blacks than in whites or Asians

Given the more accurate assessment of GFR estimates and better risk categorization, the use of the CKD-EPI equation would thus contribute to a more suitable provision of health care resources and more targeted prevention and management of CKD complications (Michels *et al.,* 2010).

4.3 Study considerations

4.3.1 Strengths of the study

A number of strengths were evident from this study, starting firstly with the care that was taken to assess the population of the study. Specific inclusion and exclusion criteria was in place to ensure all data was effectively accurate and representative of a general population. Furthermore, past studies have focused towards a patient-specific population when investigating the effects of risk markers on CKD. Evidently, this study features a non-clinical population which separates the investigation from previous related cohorts. Fundamentally, this study has helped to raise the awareness of risk in participants that may be of a non-clinical nature, which due to the silent nature of kidney disease, is a primary concern in this population.

Additionally, the collection and processing of blood samples was carried out in a structured and rigorous fashion and immediately processed as outlined by standard operating procedures. All routinely measured variables were carried out in a single operating laboratory whereby samples were stored and batch-analysed to minimise inter-assay variability.

Due to the cross-sectional nature of the study, we were unable to infer causality from the results. Despite this however, when observing aetiology, diagnosis and prognosis, observational studies prove to be much more valid than others such as randomized controlled trials (Jager *et al.,* 2007).

A strong feature or this study is that we were able to compare several frequently reported equations, both new and old, to estimate kidney equations in one heterogeneous cohort and establish which would stand as the most effective gold standard in modern clinical assessment of GFR (Michels, 2010). We were also able to study the influence of several clinical characteristics and their effects on the performance of the formulas by stratification on these parameters.

Overall findings from the study revealed a strong relationship amongst the lipid and diabetic-related variables in relation to kidney function. These findings were both expected and consistent with previous literature, further solidifying the strong relationship between these variables and their ability to predict progression of renal insufficiency.

4.3.2 Study Limitations

Study Design

Although the data presented in this thesis is regarded as representative of a general non-clinical population, the cross-sectional nature of the study design only allows us to establish an association of several factors, but not causality (Kearns *et al.,* 2013). Therefore, it would have been preferable to conduct a longitudinal study with multiple measurements of an extended period to confirm and modify the estimated prevalence of CKD risk markers (Ogna *et al.,* 2016).In addition, it is well known that renal progression rates are influenced by participant characteristics (Jafar *et al.,* 2003 and O'Hare *et al.,* 2007), therefore when targeting possible resources for CKD risk, further consideration should be given towards the variations in rates of progression across populations.

Clinical and biochemical characterization

Carrying out accurate and reliable examination of body fat percentage is one that proves to be challenging to most researchers (Khaled *et al.,* 1988). Due to the cofounding variability of participants' health state at the time of processing, several influences can have an effect on the bioelectrical impedance assessment (BIA); this includes the individuals' level of hydration, with several studies reporting as much as 2-5% decrease in body fat percentage of those that were dehydrated at the time of measurement (Girandola *et al.,* 2013). This information can have a

substantial impact from a clinical perspective as patient knowledge of over or under estimated body fat could initiate or reinforce disordered eating patterns (Saunders *et al.,* 1998). Additionally, reports have also signified the cofounding influence of other factors such as ethnicity, phase of menstruation and underlying medical conditions, on the reliability of the bioelectrical impedance assessment (Gleichauf and Roe, 1989). This evidence supports the inclination that BIA may not be the appropriate choice for body composition measurement unless specific calibration equations are developed for different groups participating in the study (Dehghan and Merchant, 2008).

A general issue facing clinical laboratories is the stability of analytes during sample processing and storage (Heins *et al.,* 1995). Lengthy contact of serum samples with cells is a common cause of spurious test results, mainly due to the ongoing metabolism of cellular constituents as well as active and passive movement of analytes between serum and cellular compartments (Boyanton and Blick, 2002). In this thesis however, we were mindful of the convoluted instability frequently associated with sample processing and therefore implemented a strategic handling process whereby fresh samples were rapidly transferred for serum separation and stored immediately to await further analysis.

One limitation that was evident form this study involved the repeated freeze-thawing of sample specimens. Previous literature tells us that continual freeze-thawing cycles are known to affect the integrity of biological samples and, more notably, to induce conformational changes in proteins that may ultimately lead to aggregation and degradation (Bhatnagar *et al.,* 2007). One study investigating freeze-thaw cycles on clinical markers found that freeze-thawing a sample more than twice could begin to compromise data quality by interfering with peak detection (Kang *et al.,* 2013). A review of laboratory errors has revealed that at least 40% of errors are made in the pre-analytical phase of processing (Kang *et al.,* 2013). Therefore, it is important to consider the influence of pre-analytical variables and how they should be considered as key factors for generating consistent experimental data. Further work would include monitoring and maintaining sample quality, as well as handling and controlling samples correctly (Sweep *et al.,* 2003).

In terms of marker specificity, concerns were raised around the reliability of the marker for glycaemic control, fructosamine. As such, it was suggested that future work would perhaps implement a more accurate parameter such as HbA_{1c} which is indicative of glycaemic control over a period of approximately 12 weeks in comparison to the 2-3 week period of fructosamine (Sacks, 2011). However, based on costing's, length and complexity of procedures, and the

accountability for fructosamine to provide a clinically accessible measure, as well as staying consistent with the time frame of the 1-week diet diaries required by participants, it was determined that HbA_{1c} would serve as unnecessary to this study (Cohen *et al.,* 2003). In addition, uncertainties were placed around the specificity of hs-CRP as it has been reported that between 20-40% of hs-CRP variability is related to genetic factors (Martinez-Calatrava *et al.,* 2007). Furthermore, several clinical and environmental factors such as age, obesity, smoking, diet, and statin treatment can modulate hs-CRP concentrations (Rojo-Martinez *et al.,* 2013). Also, to enable a more clear understanding from higher results, it was suggested that future studies should integrate a full blood count which would enable a more in-depth analysis of cause and establish whether acute infection or other sources were responsible for any abnormal readings.

In relation to the estimation of kidney function, accurate assessment is feasible through the measurement of inulin and iohexol clearance (Brandstrom *et al.,* 1998). However these methods are not widely available, are costly, and may place a burden on potential or actual participants (Botev *et al.,* 2011). Alternatively, in this study the MDRD equation with IDMS-traceable creatinine results was chosen as it is commonly used in routine clinical practice. Although, it must be said that serum creatinine poses several limitations, this includes the effects of muscle mass on reported renal function due to the production of creatinine as a result of muscle metabolism (Ikizler and Himmelfarb, 2006). Additionally, serum creatinine is commonly affected by dietary factors such as increased meat consumption prior to testing which may have influenced the results obtained (Nair, *et al.,* 2014). In previous reports, participants have been asked to refrain from eating meat 24 hours prior to testing (Mcintyre *et al.,* 2011). However, in this study it was discussed that this would place an increased burden on participants and therefore reflect results that were not generalisable to routine clinical practice (Martin *et al*., 2005).

The lack of cystatin C data is also a limitation of this study as recently, experts have proposed cystatin C as an alternative to creatinine (O'Riordan *et al.,* 2002). Moreover, this marker of GFR offers less dependency on ethnicity, sex, age, and muscle mass or protein intake when compared to creatinine and has been found to be an independent predictor of cardiovascular and overall mortality. However, when compared to GFR measurement based on the renal clearance of exogenous markers, studies have shown that eGFR is more precise when derived from both cystatin C and creatinine levels in serum (Stevens *et al.,* 2008). Furthermore, scientists have identified cystatin C as being costly and needing further validation across a broad spectrum of populations with or without CKD before it can be regarded as a potentially alternative CKD marker (Hoek *et al.,* 2003).

Study Population

Overall, target population size was met. However, the ethnic diversities within the group was not wide-spread and therefore lacked in this area. As this study involved a random recruitment process and dependent on the origin of the study (Lincolnshire) where the non-white population make up only 2.4% of the total population, it was non-surprising that we achieved this outcome (Lincolnshire Research Observatory, 2011). Nonetheless, it is well known that ethnicity has an influence on renal function and would have been an interesting factor to study further, if given a higher variability.

In addition, one limitation that was faced in this study involved the difficulties in recruiting older individuals. It is a well-established fact that age-related declines in kidney function are common across populations (Weinstein and Anderson, 2010). However, observations from the current thesis did not highlight such findings. Had we recruited a more feasible population size for this group, this may have changed the outcome of the study and reflect the supported literature on this common risk relationship.

Lastly, one factor that remained unexplored in our study population was the influence of socioeconomic status (SES). Past studies have regarded low SES as an associated risk factor for reduced renal function (Bello *et al.,* 2008). However, it must be regarded that SES on its own does not affect kidney function or share a direct link with CKD onset, but instead the related biological exposures (demographic, clinical, behavioural, and healthcare delivery systems) would contribute towards the relationship between CKD.

4.3.3 Implications for Future Research

Reflecting upon the present thesis, it is evident that introducing a screening programme for renal disease for the whole UK population is both unrealistic and likely to be counterproductive. Nonetheless, it may be possible to influence a change in the clinical setting with regards to general practioners. By opportunistically checking for renal biomarkers (particularly eGFR and ACR) in high risk populations, this will allow for the identification of CKD and provide early treatment measures for patients which can control for underlying causes such as hypertension and diabetes (Yuste *et al.,* 2013).

As previously discussed, there is a highly associated relationship between renal dysfunction and several high-risk diseases (e.g. obesity, hypercholesterolaemia, hypertension, diabetes), as well as lifestyle habits (e.g. alcohol consumption or smoking). Thus, the simplest and most effective method to limit an increase in CKD prevalence is the prevention of its risk factors. In a healthy

population, this may include the awareness of associated risk factors and knowledge on leading a healthier lifestyle (e.g. adequate dietary information and regular physical exercise). In those with pre-existing comorbidities, this may also include the addition of medical treatments such as ACE inhibitors or angiotensin II receptor blockers for chronic hypertension (Kronenberg, 2009).

Due the asymptomatic nature of early progressing renal dysfunction, CKD awareness amongst patients remains staggeringly low (Plantinga *et al.,* 2010). Timely recognition of CKD could slow disease progression, prevent complications, and reduce cardiovascular-related outcomes. Additionally, referral to a nephrologist in the early stages of disease has also shown to improve outcomes for those who progress to ESRD (Plantinga *et al.,* 2010). It is therefore critical that efforts are perused in guiding the implementation of awareness campaigns as well as ensuring adequate training in CKD awareness amongst primary care providers (PCP).

Should funding become available in the future, it would be of great interest to explore the nature of risk marker relationships and chronic kidney disease through longitudinal attempts. More specifically, the identification of several emerging risk markers such as those associated with oxidative stress (advanced oxidation protein products and circulating osteoglycin), inflammation (Interleukin-1 receptor 1 and interleukin-6), and immunohistochemical biomarkers (serum microfibrillar-associated protein-4 and cholesterol-carrying proteins) (Kronenberg, 2009). Using both traditional and novel risk markers of kidney disease, it would be possible to incorporate these parameters into various risk prediction models and determine whether their addition increases the model's predictive ability. Overall, this would give a more authentic and reliable estimation of CKD and allow for early diagnosis of disease (Upadhyay, 2015).

Lastly, the growing concerns surrounding the worldwide epidemic of end stage renal disease as well as the burden of CKD is one that must be studied further. Such concerns are linked to the increasing strain on healthcare costs and the various forms of adverse outcomes from CKD such as increased cardiovascular complications and premature mortality (Fraser *et al.,* 2015). Recent investigations show that such negative health outcomes can be prevented or at least delayed through early detection and prevention programmes (Bastos and Kirsztajn, 2011). In order to gain a greater understanding of early disease onset, it is imperative that individuals acquire a greater understanding of the burden and risk factors/markers that are associated with CKD in the community (Bello *et al.,* 2008).

If given a repeat opportunity of carrying out the present study, the following suggestions would be advised:

- Increased sample recruitment of participants and to also include a more even distribution of genders and ethnicity.
- Review of the current protocol to include morning fasting sessions.
- Further biochemical analysis to include HDL and LDL cholesterol readings as well as full blood count and blood ferritin measurements.
- Revision of literature review on present and future implications within the CKD research community.

Chapter 5.0
Conclusion

In summary, the findings from this study in combination with previous reports suggest that traditional biochemical risk markers such as those for diabetes and obesity may share an increased relationship with a reduced kidney function. While we did not see a direct correlation between the non-traditional marker, hsCRP, it was possible that this was a consequence of insufficient population numbers.

Overall, this study established that an eGFR of less than 90ml/min/1.73m2 was prevalent in 21% of the study population and that increased levels of glucose, fructosamine, triglycerides and cholesterol are components of chronic kidney disease which are independent risk factors for the development of a reduced kidney function. These findings warrant the need for future clinical evaluation to identify whether the effect of preventing and/or treating such risk factors predisposing individuals to a reduced kidney function will result in an improved renal prognosis and markedly ease the burden of kidney dysfunction in the identified population.

In addition, investigation of renal equations provided evidence of a greater accuracy in the measurement of estimated kidney function using the newer equation system, CKD-EPI, in comparison to clinically regarded formulas such as Cockcroft-Gault and MDRD. Furthermore, it was highlighted that through the use of the renal equations, correction for both age, gender and ethnicity was prominent in this study population, further ensuring the accuracy and reliability of these data sets.

Whereas the sensitivity and the accuracy of the CG formula cannot be overlooked due to the influence of weight, CKD-EPI and the MDRD equations are also difficult to calculate in clinical practice especially in our setting. However, when CKD-EPI is used bias is enhanced particularly at elevated eGFR's even though precision is not the best. Further studies are therefore, warranted prior to the generalization of these findings for patients presenting with chronic kidney disease. One theory suggests that increasing age directly affects creatinine production on the basis of a reduced overall muscle mass when compared to a younger population. This evidence was reflected in this study which highlighted a greater reduction in creatinine with increased age in our study population.

In the present thesis, we also investigated the differences amongst genders which revealed a large variance between waist: hip ratio, body fat %, systolic blood pressure and triglyceride levels. Differences in morphological characteristics of the two groups seemed to explain the reasons behind these variances. Additionally, due to the ease of interpretation of these factors, together they may prove beneficial in the assessment of risk of kidney dysfunction and related factors in men and women.

When evaluated on an additive scale, the modification of these variables by the presence of other risk factors has important clinical and public health implications with respect to CKD case findings and mass screening strategies. For example, in men with reduced renal function along with increased body fat percentage and hypertension, the probability of having CKD and/or related disease is much higher. Thus increasing the power of simple clinical measurements to identify women and men at high risk of these diseases.

Previous studies have highlighted the possibility to predict young-onset type 2 diabetes and CVD using simple and non-invasive measures of obesity. Our findings from this study may prove to be informative for prevention strategies targeting the early-life risk factors for T2DM and CVD.

As a final note, it is important to emphasize that, at present, it is still unclear which and how many patients with CKD will progress to ESRD requiring RRT. Therefore it is extremely difficult to assess whether more and more accurate controls for CKD in primary care will automatically lead to fewer cases of ESRD and RRT. As already mentioned, the probability of disease progression depends on various clinical (age, gender, co-morbidities, smoking), environmental (exposure to heavy metals, chemicals, nephrotoxic substances), and genetic factors (family history of CKD). However, it is reasonable to assume that interventions slowing or stopping disease progression by successfully treating the underlying disease are important to avoid ESRD, and might have a greater impact if implemented earlier.

Chapter 6.0
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Chapter 7.0
Appendix

Appendix A1 – Ethics and safety approval forms

EA1

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UNIVERSITY OF LINCOLN

Ethical Approval Form:

This form must be completed for each piece of research activity whether conducted by academic staff, research staff, graduate students or undergraduates. Applications by students must be endorsed by an academic member of staff acting as Principal Investigator/supervisor. The completed form must be sent to the designated Ethics Committee within the College.

Please complete all sections. If a section is not applicable, write N/A.

1 Name of Applicant	Kyle Mullan
2 School	Life Sciences
3 Position in the University	Postgraduate Research Masters
4 Role in relation to this research	Student Researcher
5 Name(s) of collaborators/co-workers and their relationship to the project (e.g. supervisor, assistant etc.)	*Name, and role in project:* 1.Dr. Adrian Slee (supervisor) 2.Dr. Ciaren Graham (supervisor) 3.Dr. Carol Rea (supervisor)
6 Brief statement of main Research Question or Project Title	**Investigating the Prevalence of Chronic Kidney Disease Risk Factors in a Mixed-Aged University Population.** Individuals at risk of CKD share many risk factors, most of which would benefit from early detection and intervention. Currently, CKD serves as a major public health problem in the UK and throughout the world. Figures from the 2010 NHS Kidney Care Association show over 1.8 million people aged over 18 are registered with CKD (stages 3-5). However, research indicates that many more go undiagnosed until the symptoms of chronic kidney failure begin to manifest, supporting the idea for earlier diagnostic

	and intervention methodologies. It is currently known that CKD and CVD share two major risk factors – diabetes and high blood pressure. Both can cause damage to the blood vessels in the kidney, preventing it from properly eliminating fluid from the body. Additionally, complications caused by CKD can also make CVD more likely to occur, this includes: - Excess calcium or phosphorus in the blood - High homocysteine levels - Systemic inflammation Since even minor loss of kidney function can drastically increase the danger of damaging the heart, several cardio-related complications can occur before the CKD is even diagnosed. **Aim/Objective:** The project proposes to carry out quantitative and qualitative assays to investigate the Incidence of chronic kidney disease risk factors in a mixed-age university population and establish the risk of development of CVD, CKD and type 2 diabetes. Body measurements, food diaries and blood and urine samples will be taken from the 150 participants recruited in the study. Samples taken from the participants will be analysed for a variety of compositions (glucose, cholesterol, triglycerides, glycated haemoglobin, creatinine and CRP). Results will be analysed along with the qualitative measurements to look for any significant correlations.
7 Ethical checklist	Does the research involve living human participants, or human tissue? **Yes** ☒ **No** ☐ *If you answered "yes", submit form EA2 for Ethical Approval.* Does the research involve living animals, or animal tissue? **Yes** ☐ **No** ☒ *If you answered "yes", submit form EA3 for Ethical Approval.* Does the research involve confidential data, or data not in the public domain? **Yes** ☒ **No** ☐ Does the project potentially put you or your collaborators at physical or psychological risk? **Yes** ☐ **No** ☒ Could the topic or results of this research be seen as illegal, or attract legal action against the University from an outside agency? **Yes** ☐ **No** ☒ Could the topic or results of this research attract unwelcome media attention, or affect the reputation or standing of the University? **Yes** ☐ **No** ☒ Could the topic, results or conduct of this research be regarded as

	offensive, immoral or destructive by some reasonable people? **Yes** ☐ **No** ☒ Does this research need to be undertaken under a relevant professional code of conduct? **Yes** ☐ **No** ☒ Are there any potential conflicts of interest in conducting this research, including financial gain for the researchers, or for individuals or external organizations affiliated with the researchers? **Yes** ☐ **No** ☒ Are there any factors inhibiting the application of the University's ethical guidelines, including those on proper treatment of data, research design and publication of results? **Yes** ☐ **No** ☒ Does the research require the approval of any external body? **Yes** ☐ **No** ☒ ***If the answer to all questions above is "No", you may complete section 8 to certify that there are no ethical issues, submit this form to the relevant Ethics Committee, and proceed with the research immediately. You accept professional responsibility for this decision, and if unsure should instead submit to the Committee.*** ***If the answer to any of the above questions is "Yes", complete the rest of the form, submit to the relevant Ethics Committee, and await approval before proceeding with the research. Answering "Yes" does not necessarily imply that the research is problematic, only the Ethics Committee needs to consider the research to ensure that it can proceed, and that the research design conforms to best practice.***
8 Self certification of Ethical Review	*Having reviewed the ethical implications of this research, I certify that there are no issues requiring Ethical Approval. I certify that the research will be carried out in compliance with the University's ethical guidelines for library/desk/laboratory/studio-based research, with Health and Safety regulations, and with all other relevant University policies and procedures. If there are any changes to the research requiring ethical clearance, I shall apply for such clearance before continuing with the research.*

	Signed: Principal Investigator ***Note.*** *This section must be endorsed by the member of academic staff responsible for the project. In the case of research by students, the supervising member of academic staff must sign. The signed form should then be submitted to the relevant Ethics Committee within the College, and the research may proceed.*
9 Does the research comply with the University's key ethical principles for library/desk/lab/studio-based research ?	**Yes** ☒ **No** ☐
	If "No", provide an ethical justification for your project and explain why you wish to continue with the research in breach of normal ethical principles:-
10 If applicable, please state the relevant professional code(s) under which the research is being conducted and confirm compliance	
11 Does this research require the approval of an external body ?	**Yes** ☐ **No** ☐
	If "Yes", please state which body:-

12 Has ethical approval already been obtained from that body ?	**Yes** ☐ **-Please append documentary evidence to this form.** **No** ☐ **If "No", please state why not:-** **Please note that any such approvals must be obtained and documented before the project begins.**
13 If there are any other ethical issues, to which the attention of the approving committee should be drawn, please state them in this section, and explain how you have taken the issues into account, so that the research should be approved. Please consult the University's ethical guidelines for advice. **Please also include here, or attach separately, a brief description of the research, to allow the approving committee to reach judgement.**	**Consent to research** All subjects taking part will be older than 18 years of age, and therefore consent will be sought directly from the participants. The participants will be recruited from The University of Lincoln. As a researcher I will be using language that is understandable to research participants in obtaining their appropriate informed consent, and in the information leaflet they will receive regarding the project. All informed consent received from the subjects would be appropriately documented prior to any research being conducted. In addition subjects will be informed on significant factors that may be expected to influence their willingness to participate such as risks involved in taking blood, which may cause discomfort and also when taking anthropometric measurements. **Right to withdraw** Participants will be informed of their right to withdraw from the research at any time without giving a reason. They will also be informed that they are free to choose not to answer any individual questions without giving a reason They will also be informed that they are free to withdraw from their questionnaire or other assessments at any time, in which case all relevant data will be destroyed and not included in the study. As a result of with drawing, as a researcher I will take special care to protect the prospective participants from adverse consequences of withdrawing from participation. **Anonymity and Confidentiality** Anonymity and confidentiality will be maintained throughout the project. Assessment results will be destroyed as soon as possible, and any identifying information will be

removed. Issues of confidentiality and anonymity will also be outlined in the consent form sent to participants No information identifying the participants will be generated and the data will be fully anonymous in the writing up of the study. Only the researcher and their supervisor will have access to personal information relating to the study. All participating subjects will be given a number that will be used instead of their name. All samples taken will be coded with the number, and only myself and the lecturers will know the subjects number. All forms and paperwork that include the names of the participants will be locked away, and any information on a computer will be password protected.

Security and Data Protection

Data will be anonymous and stored on a password-protected computer, and data collected will only be used for the purposes of this project. Data will not be left unattended on computers; Any hard copies of information such as the questionnaires and food diaries will be kept confidential and locked away.

The principle of justice

Subjects will be treated fairly; they will be equitably chosen to ensure certain individuals will not feel excluded. Risk markers in a perceived normal population will be looked at.

Providing subjects with information about the study

As a researcher, I will provide information to the subjects on the nature of the results, explaining why the research project is being carried out, and how the results will effect the conclusion. If the participants have any questions, they will be answered as well as attempting to correct any misconceptions that participants may have.

Protection of participants

Participants will not be exposed to risks greater than or additional to those encountered in their normal lifestyles. Subjects will be protected against any physical or mental harm during the investigation, as well as being asked about any factors in the procedure that may create a risk, such as pre-existing medical conditions, and will be advised of any special action that they should take to avoid risk.

Human Tissue Act

This research is not subject to the Human Tissue Act as serum is being used, and it is not regarded as relevant material for the purposes of the Human Tissue Act 2004.

APPLICANT SIGNATURE

I hereby request ethical approval for the research as described above.

I certify that I have read the University's ethical guidelines for library/desk/laboratory/studio-based research.

_____ _____

Applicant Signature **Date**

_____

PRINT NAME

FOR STUDENT APPLICATIONS ONLY –

Academic Support for Ethics

Academic support should be sought prior to submitting this form to the designated Ethics Committee within the Faculty.

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• **Undergraduate / Postgraduate Taught application**	**Academic Member of staff nominated by the School (consult your project tutor)**
• **Postgraduate Research Application**	**Director of Studies**

I support the application for ethical approval

_____ _____

Academic / Director of Studies Signature Date

_____

PRINT NAME

FOR COMPLETION BY THE DESIGNATED ETHICS COMMITTEE WITHIN THE COLLEGE

Please select ONE of A, B, C or D below:

☐ **A. Ethical approval to this research.**

☐ **B. <u>Conditional</u> ethical approval to this research.**

10 Please state the condition (inc. date by which condition must be satisfied if applicable)	

☐ **C. Ethical approval cannot be given to this research but the application is referred on to the University Research Ethics Committee for higher level consideration.**

11 Please state the reason	

☐ **D. Ethical approval cannot be given to this research and it is recommended that the research should not proceed.**

12 Please state the reason, bearing in mind the University's ethical framework, including the primary concern for Academic Freedom.	

Signature of the Chair of the designated ethics committee within the College

Signature:
_____Date:_____

Chair of_____

Key ethical guidelines for library/desk/laboratory/studio-based research

The University of Lincoln has drawn up the following key principles for researchers engaged in library/desk/laboratory/studio-based projects in order to promote high professional standards. They should be read alongside the University's Ethical Principles for Conducting Research with Humans and Other Animals, and operate as part of the University's Ethical Framework.

- *Non-falsification of data:* Researchers have an ethical obligation to refrain from tampering with data. Thus questionnaire responses, experimental observations and data analyses should not be fabricated, altered nor discarded. In addition, researchers have a responsibility to exercise reasonable care in processing data to ensure no errors affect the results.
- *Ethics of reporting research:* Researchers are obliged to give full and proper attribution of ideas: presenting the words, data or ideas of another person as your own without properly citing them amounts to plagiarism. This is not only misconduct but can also be an infringement of copyright, amounting to theft of intellectual property.

- *Ethics and research design:* Researchers should be open to a range of methods: failure to consider and evaluate alternative methods and tools for the collection of data may be regarded as too overtly biased. All appropriate steps should be taken to ensure that no samples are obtained from unethical sources e.g. illegal databases; unregistered suppliers of samples from humans or other animals.
- *Authorship credit:* Only those researchers who are significant contributors to a research project should be given authorship credit. A "significant contributor" might be described as a person playing a major role in conceptualising, analysing or writing the final document. Ideally, all those involved in the research project should decide upon the order of authorship. Usually, the first author is the one who has made the biggest contribution.
- *Conflict of interest:* Researchers should be aware of the potential influence of personal or commercial interests on their work and take all practical measures to ensure that information is presented without distortion.
- *The principle of beneficence:* Researchers are required to protect individuals by seeking to maximise anticipated benefits and minimise possible harms. It is therefore necessary to examine carefully the design of the study and its risks and benefits including, in some cases, identifying alternative ways of obtaining the benefits sought from the research. Research risks must always be justified by the expected benefits of research.
- *Professional codes:* Researchers should undertake research legally and in accordance with any relevant professional codes of conduct.
- *Personal information:* Researchers should anonymise information which relates to individuals when they have not obtained informed consent, unless there is a clear justification to the contrary. They should also be aware of the impact of wider public dissemination of their work and the impact this might have on any individual or group of individuals. If it is anticipated that it might cause distress, it is essential to demonstrate that the benefits outweigh this risk.

9 **Statement of the ethical issues** **involved and how they are to** **be addressed –including a risk assessment of the project based on the vulnerability of participants, the extent to which it is likely to be harmful and whether there will be significant discomfort.** **(This will normally cover such issues as whether the risks/adverse effects** **associated with the project have**	**Consent to research** All subjects taking part will be older than 18 years of age, and therefore consent will be sought directly from the participants. The participants will be recruited from The University of Lincoln. As a researcher I will be using language that is understandable to research participants in obtaining their appropriate informed consent, and in the information leaflet (Participant Information Sheet) they will receive regarding the project. All informed consent received from the subjects would be appropriately documented prior to any research being conducted. In addition subjects will be informed on significant factors that may be expected to influence their willingness to participate such as risks involved in taking blood, which may cause discomfort and also when taking anthropometric measurements. **Right to withdraw** Participants will be informed of their right to withdraw from the research at any time without giving a reason. They will also be informed that they are free

EA2

Ethical Approval Form:

Please word-process this form, handwritten applications will not be accepted

UNIVERSITY OF LINCOLN

This form must be completed for each piece of research activity whether conducted by academic staff, research staff, graduate students or undergraduates. The completed form must be approved by the designated authority within the College.

Please complete all sections. If a section is not applicable, write N/A.

1 Name of Applicant	Kyle Mullan	
	School: Life Sciences	College: College of Science
2 Position in the University	Post Graduate Research Masters	
3 Role in relation to this research	Student Researcher	
4 Brief statement of main Research Question	**Investigating the Prevalence of Chronic Kidney Disease Risk Factors in a Mixed-Aged University Population.**	
5 Brief Description of Project	**Investigating the Prevalence of Traditional and Novel Chronic Kidney Disease Risk Factors in a Mixed-Aged University Population.** Individuals at risk of chronic kidney disease (CKD) share many risk factors, most of which would benefit from early detection and intervention. Currently, CKD is a major public health problem in the UK and throughout the world. Figures from the 2010 NHS Kidney Care Association show over 1.8 million people aged over 18 in the UK are registered with CKD (stages 3-5). However, research indicates that many more go undiagnosed until the symptoms of chronic kidney failure begin to manifest, supporting the idea for earlier diagnostic and intervention methodologies.	
been dealt with and whether the benefits of research outweigh the	to choose not to answer any individual questions without giving a reason They will also be informed that they are free to withdraw from their questionnaire or other assessments at any time, in which case all relevant data will be	

	It is currently known that CKD and CVD share two major risk factors – diabetes and high blood pressure. Both can cause damage to the blood vessels in the kidney, preventing it from properly eliminating fluid from the body. Additionally, complications caused by CKD can also make CVD more likely to occur, this includes; excess calcium or phosphorus in the blood, anemia, high homocysteine levels, systemic inflammation. Since even minor loss of kidney function can drastically increase the danger of damaging the heart, several cardio-related complications can occur before the CKD is even diagnosed. **Aim/Objective:** The project proposes to carry out quantitative and qualitative measures to investigate the incidence of CKD risk factors in a mixed-age university population and establish the risk of development of CVD, CKD and type 2 diabetes. Body measurements (height, weight, body fat percentage, waist and hip circumference), haemodynamic function (heart rate and blood pressure), food diaries and blood samples will be taken from the 150 participants recruited in the study. Samples taken from the participants will be analysed for a number of variables (glucose, cholesterol, triglycerides, glycated haemoglobin, creatinine and CRP). Results will be analysed along with the qualitative measurements to look for any significant correlations and group analyses performed using SPSS.	
	Approximate Start Date: November 2014	Approximate End Date: August 2014
6 Name of Principal Investigator or Supervisor	Dr. Adrian Slee Dr. Ciaren Graham Dr. Carol Rea	
	Email address: cgraham@lincoln.ac.uk crea@lincoln.ac.uk aslee@lincoln.ac.uk	Telephone: 6897 6819

7 Names of other researchers or student investigators involved	Nikita Gug
8 Location(s) at which project is to be carried out	Joseph Banks Labs (Science Building), and MHT Joseph Banks Laboratories Beevor Street, University of Lincoln, Lincoln LN6 7DL MC3133 Dry Lab
risks)	destroyed and not included in the study. As a result of with drawing, as a researcher I will take special care to protect the prospective participants from adverse consequences of withdrawing from participation. **<u>Anonymity and Confidentiality</u>** Anonymity and confidentiality will be maintained throughout the project. Assessment results will be destroyed as soon as possible, and any identifying information will be removed. Issues of confidentiality and anonymity will also be outlined in the consent form sent to participants No information identifying the participants will be generated and the data will be fully anonymous in the writing up of the study. Only the researcher and their supervisor will have access to personal information relating to the study. All participating subjects will be given a number, that will be used instead of their name. All samples taken will be coded with the number, and only myself and the lecturers will know the subjects number. All forms and paperwork that include the names of the participants will be locked away, and any information on a computer will be password protected. **<u>Security and Data Protection</u>** Data will be anonymous and stored on a password-protected computer, and data collected will only be used for the purposes of this project. Data will not be left unattended on computers; Any hard copies of information such as the questionnaires and food diaries will be kept confidential. **<u>The principle of justice</u>** Subjects will be treated fairly; they will be equitably chosen to ensure certain individuals will not feel excluded. Risk markers in a perceived normal population will be looked at.

	Providing subjects with information about the study As a researcher, I will provide information to the subjects on the nature of the results, explaining why the research project is being carried out, and how the results will effect the conclusion. If the participants have any questions, they will be answered as well as attempting to correct any misconceptions that participants may have. **Protection of participants** Participants will not be exposed to risks greater than or additional to those encountered in their normal lifestyles. Subjects will be protected against any physical or mental harm during the investigation, as well as being asked about any factors in the procedure that may create a risk, such as pre-existing medical conditions, and will be advised of any special action that they should take to avoid risk. **Human Tissue Act** This research is not subject to the Human Tissue Act as serum is being used, and it is not regarded as relevant material for the purposes of the Human Tissue Act 2004.

Ethical Approval From Other Bodies

10 Does this research require the approval of an external body ?	**Yes** ☐ **No** ☒ (x)
	If "Yes", please state which body:-

11 Has ethical approval already been obtained from that body ?	**Yes** ☐ **-Please append documentary evidence to this form.** **No** ☐ **If "No", please state why not:-** **Please note that any such approvals must be obtained and documented before the project begins.**

APPLICANT SIGNATURE

I hereby request ethical approval for the research as described above.

I certify that I have read the University's ETHICAL PRINCIPLES FOR CONDUCTING RESEARCH WITH HUMANS AND OTHER ANIMALS.

_____ _____

Applicant Signature **Date**

_____

PRINT NAME

FOR STUDENT APPLICATIONS ONLY –

Academic Support for Ethics

Academic support should be sought prior to submitting this form to the designated Ethics Committee within the Faculty

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dergraduate / Postgraduate Taught application **Academic Member of staff nominated by the School (consult your project tutor)**

- **Postgraduate Research**
- **Application**

Director of Studies

I support the application for ethical approval

_____ _____

Academic / Director of Studies Signature Date

_____

PRINT NAME

FOR COMPLETION BY THE DESIGNATED ETHICS COMMITTEE WITHIN THE COLLEGE

Please select ONE of A, B, C or D below:

☐ **A. Ethical approval is given to this research.**

☐ **B. <u>Conditional</u> ethical approval is given to this research.**

Please state the condition (inc. date by which condition must be satisfied if applicable)	

☐ **C. Ethical approval cannot be given to this research but the application is referred on to the University Research Ethics Committee for higher level consideration.**

Please state the reason	

☐ **D. Ethical approval cannot be given to this research and it is recommended that the research should <u>not</u> proceed.**

Please state the reason, bearing University's ethical framework, primary concern for Academic	

Signature of the Chair of the designated Ethics Committee within the College

_____ _____

Signature **Date**

Chair of_____

UNIVERSITY OF LINCOLN

Science Building

School of Life Sciences

COSHH ASSESSMENT FORM

Name	***Nikita Gug & Kyle Mullan***
Name of Supervisor	Ciaren Graham **Carol Rea** **Adrian Slee**
Title of Project	Horiba BioAnalyser
Procedure	Measurement of Cholesterol, Glucose and Trigylcerides

BRIEF DESCRIPTION OF PROCEDURE

Measurement of Glucose: Enzymatic method (hexokinase) Hexokinase catalyses the phosphorylation of glucose in the sample by ATP producing ADP and glucose-6-phosphate. Glucose-6-phosphate is oxidised to 6-phosphogluconate with the reduction of NAD+ to NADH by G-6-PDH. The amount of NADH formed is proportional to the concentration of glucose in the sample and can be measured by the increase in absorbance at 340 nm.

Measurement of Cholestrol. ''CHOD-PAP'': enzymatic photometric test. Determination of cholesterol after enzymatic hydrolysis and oxidation. The colorimetric indicator is quinoneimine which is generated from 4-aminoantipyrine and phenol by hydrogen peroxide under the catalytic action of peroxidase (Trinder's reaction).

Measurement of triglycerides. A series of enzymatic reactions, again as in the test for cholesterol, quinoneimine is produced which is measured photometrically

Measurement of glycated haemoglobin (HbA1c). Whole blood samples are pre-treated with the Haemolysis Reagent to lyse the red cells and hydrolyze the haemoglobin chain. Total Haemoglobin is converted into alkaline haematin in the alkaline solution of a non-ionic detergent. HbA1c is measured from the hemolysate by a latex enhanced turbidimetric immunoassay.

Measurement of high-sensitivity C-reactive protein (Hs-CRP). When an antigen-antibody reaction occurs between CRP in a sample and anti-CRP antibody which has been sensitized to latex particles, agglutination results. This agglutination is detected as an absorbance change, with the magnitude of the change being proportional to the quantity of CRP in the sample. The actual concentration is then determined by interpolation from a calibration curve prepared from calibrators of known concentration.

Measurement of Creatinine. An in vitro diagnostic assay for the quantitative in vitro determination of creatinine in human serum, plasma and urine based on an enzymatic method using a multi-step approach ending with a photometric end-point reaction. It is composed of a bi-reagent cassette (R1= 22 mL;R2= 8 mL).

All reagents come supplied ready for use; the cap of the reagent cassette is removed and placed in the refrigerated ABX Pentra 400 reagent compartment.

TABLE 1. CHEMICALS USED

	Chemical name and concentration (including constituents of mixtures) Or micro-organism, dust etc	Maximum quantity present	Hazard category (Or micro-organism category)	Physical form (Gas, liquid, solid)	Hazard rating (Low, Medium, High, Extreme)
1	**Pentra Glucose HK CP** is ready-to-use. Composition. Reagent 1 Pipes Buffer, pH 7.60 100 mmol/l NAD 3.8 mmol/l ATP 2.2 mmol/l Sodium azide < 0.1 % Reagent 2: Hexokinase 8500 U/l G-6-PDH 8500 U/l Magnesium sulphate 20 mmol/l Sodium azide < 0.1 %	Reagent 1 : 56 ml Reagent 2 : 14 ml	Toxic. Irritant	Liquid	Medium
2	**ABX Pentra Cholesterol CP** is ready-to-use. Composition. Good's buffer pH 6.7 50 mmol/l, Phenol 5 mmol/l 4-Aminoantipyrine 0.3 mmol/l, Cholesterol esterase (CHE) 200 U/l, Cholesterol oxidase (CHO) 50 U/l, Peroxidase (POD) 3 kU/l Sodium azide 0.95 g/l	90 ml	Toxic. Irritant	Liquid	Medium

3	**ABX Pentra Triglycerides CP** is ready-to-use. Composition. Pipes free acid 50 mmol/l Sodium hydroxide 3.36 g/l Triton X-100 1 ml/l Magnesium salt 14.8 mmol/l p-chlorophenol 2.69 mmol/l ATP 3.14 mmol/l Sodium azide 7.99 mmol/l, Potassium ferrocyanide 9.94 mol/l, 4-aminoantipyrine 0.31 mmol/l Lipoprotein lipase 1.90 U/l Glycerokinase 0.5050 KU/l Glycerol phosphate Oxidase 4.15 KU/l Peroxidase 0.4950 KU/l Distilled water qs 1l/l	90 ml	Toxic. Irritant	Liquid	Medium
	ABX Pentra HbA1c WB	90ml	Toxic. Irritant	Liquid	Medium
	ABX Pentra Creatinine CP	90ml	Toxic. Irritant	Liquid	Medium
	ABX Pentra High Sensitive C-Reactive Protein	90ml	Toxic. Irritant	Liquid	Medium

Ex = Explosive, To = Toxic, Co = Corrosive, Ha = Harmful, Ir = Irritant, Fl = Flammable, HF = Highly Flammable, Ox = Oxidising, Ca = Carcinogenic, Ter = Teratogen, Mu = Mutagen

SPECIFIC DETAILS FOR HIGH RISK CHEMICALS

TABLE 2. EXPOSURE POTENTIAL

Chem no.	Exposure Potential (Low, Med, High)	Possible Route of Entry (tick)				Frequency of Use	Length of exposure
		Skin	Eyes	Inhal.	Ingest.		
1	Low	x	x	x	x	Once	<10mins
2	Low	x	x	x	x	Once	<10mins
3	Low	x	x	x	x	Once	<10mins
4	Low	x	x	x	x	Once	<10mins
5	Low	x	x	x	X	Once	<10mins
6	Low	x	x	x	x	Once	<10mins
7							
8							
9							
10							

Containment Level for Procedure:	**1**

Containment Level	Control Measures
1	Laboratory coat must always be worn, safety spectacles and gloves are recommended. Work may be carried out on the bench
2	Laboratory coat and gloves must be worn. Work should be carried out in a properly tissue culture hood.
3	Laboratory coat, safety spectacles and gloves must be worn. Work should be carried out in a properly functioning fume cupboard. Additional safety measures may be required. Full details to be entered below.

PERSONAL PROTECTIVE EQUIPMENT AND ENGINEERING CONTROLS TO BE USED DURING PROCESS

Laboratory coat must always be worn, safety spectacles and gloves are recommended. Work may be carried out on the bench

(Protective clothing, gloves (state material), eye/face protection, dust mask, respirators, fume cupboard, class II cabinet etc.)

FIRST AID

Eyes:	Check for and remove contact lenses and flush with copious amounts of water; assure adequate flushing by separating the eyelids with fingers; call a physician
Skin:	Generally the product does not irritate the skin. However flush with copious amounts of water; remove contaminated clothing and shoes; call a physician
Inhalation:	Supply fresh air; consult doctor in case of complaints
Ingestion:	If swallowed, wash out mouth with copious amounts of water; call a physician

WASTE DISPOSAL

Solvents used are divided into chlorinated and unchlorinated waste and are placed in solvent waste bottles ready for safe disposal. Oils and fats are disposed of carefully in waste bottles. It is important they are NOT disposed of down the sink. **Some chemicals can go down the sink if they are diluted with plenty of running water.** **Important to always check the method of disposal on the material safety data sheet (MSDS) or seek advice from technical staff.**

Assessor(s)	Nikita Gug & Kyle Mullan
Date:	22/10/2014

Checked and approved by	
Date:	

Appendix A2 –Participant information form

Participant Information Form

Participant Number _____

To investigate the potential risk factors associated with the development of cardiovascular disease and metabolic diseases such as type 2 diabetes. This will be performed in a mixed university population (i.e. students and staff).

Introductory outline

- **This study is part of MRes research studies for Nikita Gug and Kyle Mullan, Postgraduate Researchers, Biomedical Science, School of Life Sciences. This study is being supervised by Dr. Adrian Slee, Dr. Ciaren Graham and Dr. Carol Rea.**

What is the purpose of this study?

- To investigate the potential risk factors associated with the development of cardiovascular disease (CVD), metabolic diseases such as type 2 diabetes mellitus (T2DM) and related metabolic syndrome (MetS) and

chronic kidney disease (CKD). This will be performed in a mixed university population (i.e. students and staff).

Am I suitable for this study (Inclusion, exclusion criteria)

- To participate in this study, the participants need to be over the age of 18 years and be able to provide written consent. If they are pregnant, unfortunately they cannot take part in this study. Additionally, if they have been previously diagnosed with diabetes, cardiovascular problems or kidney disease they cannot take part. If they have a clinically diagnosed disorder, they should consult the principal investigators and find out whether they can take part in this study. If the participants have a fitted electrical device you will not be able to take part in the study. If you would like to take part, we would like you to complete a five day food diary and a general questionnaire. We would also like to take some non-invasive body composition measurements including height, weight, body mass index, body circumferences (waist, hip) and percentage body fat using a bio-electrical impedance device. Blood pressure and heart rate will also be taken in the rested state. Finally, we would like to take a small sample (2 x 4ml) of blood from your arm. **Please note that a bio-electrical impedance for measuring fat mass will be used, therefore if you have any electrical device fitted such as a cardiac pacemaker, unfortunately we will not be able to include you in this study.**

Days of Research

- **Week 11 – Wednesday 3rd December 2014**
- **Week 12 - Monday 8th December and Tuesday 9th December 2014**
  Location: MHT Building, Dry Lab MC3133 and Science Building, SB012

Participants will only be required to attend once and a time and date will be allocated.

Do I have to take part?

- **No.** You can withdraw from the study at any time without a reason. You will also be informed that you are free to choose not to answer any

individual questions without giving a reason, and you are free to withdraw from the questionnaire or other assessments at any time, in which case all relevant data will be destroyed and not included in the study. If you have any questions, please do not hesitate in contacting the principal investigators using the details at the bottom of this page. Please note ethical approval has been permitted to allow this study to take place and has been passed through the school of life sciences.

Are there any risks/hazards I should be aware of before taking part?

- All tests are safe and non-invasive. There is minimal risk and harm for participants. With regards to blood phlebotomy, blood will be taken by a trained phlebotomist. The health and safety of each participant is of great importance, and at no point will it be compromised. **The bioelectrical impendence should not be performed on any individual with a pacemaker, fitted electrical medical device or pregnant.**

Summary of additional forms prior to the research

- Participant Information Sheet
- Consent form
- Questionnaire

Summary of measurements being taken

- Height
- Weight
- Body Mass Index
- Waist Circumference
- Hip Circumference
- Bioelectrical impedance assessment (assessment of body composition and fat mass)
- Resting blood pressure and heart rate
- Blood measures including; cholesterol, blood glucose (including

glycated haemoglobin HbA1c), triglyceride, creatinine, C-reactive protein, serum ferritin, serum Iron.

The diet diary

- A five day diet diary needs to be completed, and should include a log of everything you consume. This study requires the food diary to be started on a Thursday, and finished on a Monday at 23.59pm.
- Participants should use a paper diary to record and track everything consumed. Your researcher will give you a paper diary.
- This study relies on your honesty when logging your diet diary. Please do not feel under any pressure to change what you eat because you are filling out the diary.

What will happen with my results?

- All participant information and data will be completely confidential and participants anonymised and given a unique code. All forms and paperwork will be kept safe within a lockable cabinet within a lockable room at the university. All electronic data will be kept on password encrypted computer. Only the principal investigators and supervisors will have access to participant information. Specific data from participants such as questionnaire and diet diary information will be analysed alongside other participants, correlations and significant relationships will be established and aim to publish results.

Your participation is appreciated and please feel free to contact us further.

Contact Information

Principal investigators – Nikita Gug & Kyle Mullan

Email – ngug@lincoln.ac.uk & kmullan@lincoln.ac.uk

Supervisors:

Dr. Adrian Slee – aslee@lincoln.ac.uk

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Dr. Carol Rea- crea@lincoln.ac.uk

Appendix A3–Participant consent form

Participant Consent Form

Participant Number _____

An Investigation into the Prevalence of Traditional and Non-Traditional Risk Factors for Cardiovascular and Related Metabolic Diseases

Thank you for showing interest in the study. Please ensure that you have read through and understand all the information given to you in the participant information pack. If you have any questions, please do not hesitate to contact the researcher before signing this form. Your participation is greatly appreciated by the primary researcher and all the other participants and researchers involved.

Please tick the appropriate box for each statement below:

- I confirm that I have read and fully understood the information on the research information sheet. I have had the chance to ask questions and have received satisfactory answers.
  Agree □ **Disagree** □

- I am aware that a bioelectrical impendence is used in the research and it involves the submission of a small electric current through the body. I can also confirm that I do not have any electrical medical devices on my person (e.g. cardiac pacemaker).
  Agree □ **Disagree** □

- I understand that researchers may use data collected during the study, I give permission for these individuals to have access to my data, and understand the results from the study could go towards a student publication; however your identity will be kept anonymous. Only the researchers and supervisors will have access to personal information relating to the study.
  Agree □ **Disagree** □

- I am aware that 8ml of blood will be taken from my arm using a syringe and needle, and blood will be tested for typical risk markers for cardiovascular disease, type 2 diabetes and chronic kidney disease (e.g. blood glucose, triglyceride, cholesterol and creatinine levels).
  Agree □ **Disagree** □

- I am aware that weight, height, hip and waist circumference, skin fold thickness, body composition, body fat percentage and blood pressure measurements will be taken.
  Agree □ **Disagree** □

- I agree to complete a five day food diary.
  Agree □ **Disagree** □

- I am aware that all data will be kept confidential and stored in a secure and safe manner.
  Agree □ **Disagree** □

- I understand that my participation is voluntary and that I am free to withdraw at any time without giving a reason.
  Agree □ **Disagree** □

If you have selected 'Disagree' to any of the statements above, you may not be eligible to participate in this study and you should speak to the primary researcher.

By signing this form you are aware of what the study entitles and you confirm that all questions on this form have been honestly answered.

Participant name …….

Participant signature ……

Date ……

Primary researcher name ……

Primary researcher signature ……..

Date …….

Appendix A4–Participant information booklet

Chronic Kidney Disease and associated risk factors

Project Research

Chronic Kidney Disease (CKD)

The facts

- There are currently 1.8 million people registered with CKD (stages 3-5) in the UK, a figure that continues to rise.
- Recent studies indicate that many individuals go undiagnosed with CKD until symptoms begin to manifest at a later age.

What is it?

- CKD tends to consist of a gradual loss of renal function, which over time, can lead to end stage renal disease (ESRD).
- The disease process is initiated when non-operational scar tissue replaces injured nephrons (functional units in the kidneys).

Who gets it?

- Generally, CKD prevalence tends to rise with increasing age. However, several conditions can initiate the slow, progressive destruction of the nephrons. This includes:
  - Diabetes mellitus (types 1 + 2)
  - Hypertension - Urine obstructions
  - Renal disease - Sickle cell disease

Associated risk factors

The facts

- Cardiovascular disease (CVD) Is the UK's biggest cause of death with an estimated 74,000 deaths per year (200 people per day).

What is it?

- CVD is disease of the heart and/or blood vessels which is normally caused by the build-up of a fatty plaque known as atheroma.
- Increased atheroma within the body causes blood vessels to become narrowed; which in turn puts a large amount of pressure on the vessels and heart, leading to a number of consequences such as heart attacks and strokes.

Who gets it?

- CVD is more common in individuals with:
  - Obesity
  - Poor exercise
  - Poor diet
  - Heavy smoking
  - Chronic Kidney disease

What is the purpose of this research?

Previous studies have indicated a high link between CKD and CVD, thus it is important we establish this link and try to formulate preventative strategies by testing for well known risk factors for these diseases.

This study will help to better understand these pre-disposing risk factors and help focus the importance of better diagnostic and preventative methods at an earlier stage of disease.

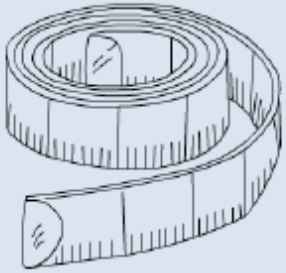
What will happen?

A series of tests will be carried out; this will involve taking measurements from you and also taking blood from you.

A highly trained phlebotomist will be taking your blood and all required safety checks will be in place to ensure your safety throughout the procedure.

Following this your blood will be separated using a spinning machine known as a centrifuge. The separated serum will then be analysed by our bio-analyser machine which will test for and identify several key measurements (see back page).

What measurements will be taken?



During the study, you will be asked for a series of measurements; this includes the following:

- Weight
- Height
- Body mass index
- Blood pressure
- Waist: hip ratio
- Bioelectrical impedance assessment (estimation of body fat %).

After blood samples have been taken their content will be measured, this involves the measurement of:

- Triglycerides
- Cholesterol
- Glucose
- Creatinine
- C-reactive protein
- Fructosamine

Blood samples will NOT be stored once the research has finished.

Food diary

For this investigation you will also be asked to make a 5 day food diary, materials will be provided to you during the body measurement phase.

Safety

Your safety is of the highest importance, significantly, the bioelectrical impedance assessment involves sending a small shock of electrical currents through your body and therefore it is crucial that you inform the investigator of any electrical implants (pacemaker) or pregnancy.

Contact details

To get in contact with me or the university, details are as followed:

Kyle Mullan (Masters Research student)

Email – kmullan@lincoln.ac.uk

Research supervisor:

Dr Carol Rea

Email – Crea@lincoln.ac.uk

Office phone - 01522886880

Subject Information

University Of Lincoln

Masters Research Project

"Investigating the prevalence of traditional and novel risk factors for chronic kidney disease in a mixed–age university population."

This information leaflet will tell you everything you need to know about the research being carried out and where you come into it.

Appendix A5–Participant lifestyle questionnaire

Questionnaire

Participant Number _____

An Investigation into the Prevalence of Traditional and Non-Traditional Risk Factors for Cardiovascular and Related Metabolic Diseases

Personal Information

1. Age _____

2. What is your occupation?

Student ☐
Lecturer ☐
Other (please state)
_____

3. Contact telephone number:_____

4. Contact email:_____

5. Sex:

Male ☐
Female ☐
Other (please state) _____

6. Ethnicity?

White ☐
Black ☐
Asian ☐

Medical History

7. Do you currently suffer from any medical conditions?

No ☐
Yes ☐
If yes, please state below:

8. Do you regularly take any prescription medication?

No ☐
Yes ☐
If yes, please state below:
_____

9. Have you had any surgery in the past year?

No ☐
Yes ☐
If yes, what was the surgery for?
_____
_

Other (please state): _____

Medical History Continued

11. Do you have any of the following? (if known)

- **Chest pain during resting activity**
  No ☐ Yes ☐
- **Chest pain during physical activity**
  No ☐ Yes ☐
- **Light headedness**
  No ☐ Yes ☐
- **Unusual shortness of breath**
  No ☐ Yes ☐
- **Cramping pains in legs or feet**
  No ☐ Yes ☐

12. Do you or any of your immediate family have any of the following conditions:

- **Heart problems**
  Self ☐ Immediate Family ☐
- **Diabetes (Type 1)**
  Self ☐ Immediate Family ☐
- **Diabetes (Type 2)**
  Self ☐ Immediate Family ☐
- **Chronic Kidney Disease**
  Self ☐ Immediate Family ☐
- **Stroke**
  Self ☐ Immediate Family ☐
- **Anaemia**

10. Are you pregnant now, or have you given birth in the past 6 months?

Yes ☐
No ☐

Lifestyle

13. Have you ever received a flu vaccination?

Yes ☐
No ☐

14, If you answered 'Yes' to question 13, when?

_____

15. How many cigarettes do you smoke per day?

I do not smoke ☐
Less than 10 ☐
10-20 ☐
20-30 ☐
>30 ☐
If other, please state (Includes nicotine patches, pipes, cigars and e-cigs):
_____

16. If you answered 'I do not smoke' to question 15, have you ever smoked?

No ☐
Yes ☐
If yes, how long ago and approximately how many?
_____

17. How many units of alcohol do you consume in an average week? Please use the table as a unit reference:

Alcoholic beverage	Number of units
Small glass of wine (175ml)	1.5
Large glass of wine (250ml)	3

Self ☐ Immediate Family ☐

- **High Blood Pressure**

  Self ☐ Immediate Family ☐

Pint of beer/lager/cider (568ml)	3
Single shot of spirit (35ml)	1

_____ Units

Lifestyle continued

18. Please list your average daily activities at home including the duration (e.g. housework, gardening, caring for children):

Activities	Time (mins)

19. Please list your average daily activities at work (e.g. manual handling, desk work, lecturing, attending lectures):

Activities	Time (mins)

20. How do you travel to university/work? (More than one can apply):

Walk ☐
Car ☐
Cycle ☐
Train ☐
Bus ☐
Other (please state) _____

21. How many times a week do you take part in structured physical activities for more than 30 minutes at a time?

0 ☐
1-2 ☐
3-4 ☐
5-6 ☐
More than 6 ☐

22. When you take part in structured physical activity, how intense is your workout?

E.g:

- **Light (not really sweating)**
- **Moderate (out of breath and slightly sweating)**
- **Intense (out of breath and not able to hold a conversation).**

I do not take part in physical activity ☐
Light ☐
Moderate ☐
Intense ☐

23. Would you like to be more active/exercise more frequently?

Yes ☐
No ☐

Lifestyle continued 2

24. If yes, why do you not?

Not enough money ☐
Not enough time ☐
No energy ☐
Other (please state)_____

25. How deep a sleeper are you?

Light Sleeper (e.g. easily woken up by noises, struggle to fall asleep) ☐
Medium Sleeper (Sometimes woken by noises but not often, fall asleep relatively easily) ☐
Heavy Sleeper (Rarely woken up by noises, fall asleep easily) ☐

26. On average what time do you go to sleep?

Before 9pm ☐
9-11pm ☐
11pm-1am ☐
1-3am ☐
After 3am ☐

27. How many hours of sleep do you have on average per night?

More than 8 hours ☐
6-8 hours ☐
4-6 hours ☐
Less than 4 hours ☐

28. If stress is defined as feeling irritable, anxious, or having sleeping difficulties, how often have you felt stress as a result of conditions at home or at work?

Never experienced stress ☐
Experience some periods at home or at work ☐
Experience several periods at home or at work ☐
Experience permanent stress at home or at work ☐

29. In your immediate family, what is the highest level of education achieved?

None ☐
Don't Know ☐
GCSE/O levels (or equivalent) ☐
A Levels ☐
Degree ☐
Postgraduate Degree ☐
Other (please state) _____

Diet

30. Are you on any special diet?

No ☐
Vegetarian ☐
Vegan ☐
Gluten Free ☐
Dairy Free ☐
Other _____

31. Would you consider yourself to have a healthy diet?

Yes ☐

No ☐

32. Do you normally eat breakfast in the morning?

Yes ☐
No ☐

33. Do you snack in-between meals?

Yes ☐
No ☐

34. Do you think you eat more than you need to or eat past the point of fullness?

Yes ☐
No ☐

35. How often do you eat a takeaway?

More than once a week ☐
Once a week ☐
Once a month ☐
Once every few months ☐
Never ☐

36. Do you skip meals?

Yes ☐
No ☐

37. How often do you eat vegetables, fruit?

Everyday ☐
Not everyday ☐

Thank you for completing this questionnaire.
Please hand it back to the principal investigator (Kyle), and if you have any questions, feel free to contact us.